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**Brief Report**

---

- 1 - 3**      **The treatment effect of the burn wound healing by electrolyticreduction ion water lotion.**  
*Tetsuo Shu, Masahiro Okajima, Ken-ichi Shimokawa, Fumiyoshi Ishii*

**Original Articles**

---

- 4 - 8**      **A study of the relationship between mental health and menstrual abnormalities in female middle school students from postearthquake Wenchuan.**  
*Xiaoxia Liu, Yanfang Yang, Ping Yuan, Xun Zhang, Ying Han, Yi Cao, Guoyu Xiong*
- 9 - 16**      **Rapid increase in Japanese life expectancy after World War II.**  
*Yasuo Sugiura, Young-Su Ju, Junko Yasuoka, Masamine Jimba*
- 17 - 24**      **Bioavailability and biological activity of liquisolid compact formula of repaglinide and its effect on glucose tolerance in rabbits.**  
*Boushra M. El-Houssieny, Lobna. F. Wahman, Nadia M. S. Arafa*
- 25 - 30**      **Evaluation of estrogen receptor *alpha*, estrogen receptor *beta*, progesterone receptor, and cKIT expression in desmoids tumors and their role in determining treatment options.**  
*Gabriel A. C. Santos, Isabela W. Cunha, Rafael M. Rocha, Celso A. L. Mello, Gustavo C. Guimarães, José H. Fregnani, Ademar Lopes*
- 31 - 36**      **The healing effect of electrolytic-reduction ion water on burn wounds.**  
*Masahiro Okajima, Ken-ichi Shimokawa, Fumiyoshi Ishii*

**Letter**

---

- 37 - 38**      **Compliance with the triage protocols.**  
*Jean-Pierre Tourtier, Laurette Mangouka, Sthpane de Rudncki, Delphine Lemoullec*

# CONTENTS

(Continued)

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## Guide for Authors

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## Copyright

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### Cover Photo of this issue

#### **Bristol University, Bristol City, UK**

Bristol University located at the center of Bristol City, UK, was founded in 1876 and received a Royal Charter in 1909. The university has a relevantly high level of the academic standard and is regarded as one of the best universities in UK. The university is also famous for the quite impressive Gothic buildings.

( Photo by Ruoyan Gai )



## Brief Report

# The treatment effect of the burn wound healing by electrolytic-reduction ion water lotion

Tetsuo Shu<sup>1,\*</sup>, Masahiro Okajima<sup>2</sup>, Ken-ichi Shimokawa<sup>2</sup>, Fumiyoshi Ishii<sup>2,\*\*</sup>

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### Summary

A 2-year-and-4-month-old girl suffered a burn to the neck due to boiling water. She was examined at another hospital, and recommended to undergo dermatoplasty. Thirteen days after injury, she consulted our hospital. A wide skin defect was observed around the neck, and a third-degree burn was diagnosed. Conservative treatment using electrolytic-reduction ion water (ERI) lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment was performed without dermatoplasty. Treatment of the burn was started with the application of ERI lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment to the wound 3 times a day combined with wrap therapy. The lysis of necrotic tissue and granulation began 1 week after the beginning of treatment. After 2 weeks, the necrosed skin had completely lysed, satisfactory granulation tissue began to form and blood supply improved. Regeneration of the skin was noted at 1-3 months after the beginning of treatment. Complete epithelialization was observed after 4 months, but hypertrophic cicatrization and pigmentation began to occur. After 26 months, capillary growth was observed, cicatrix became inconspicuous, pigmentation disappeared, and the burn almost completely healed. In conclusion, in this patient, the process of tissue repair after burn injury progressed smoothly, and healing was achieved without leaving hypertrophic cicatrix, keloid scar, or pigmentation. Our experience suggested that even third-degree burns can be treated using only external therapy with ERI lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment without dermatoplasty.

**Keywords:** Electrolytic-reduction ion water, burn wound, moist wound healing, conservative treatment, wrap therapy

### 1. Introduction

There are still many instances of burn injury caused by boiling water (1). Particularly, children with immature skin tend to suffer deeper and severer burns compared with adults. On treating third-degree burns, mesh skin grafting and patch dermatoplasty are often performed after debridement to prevent contracture of the injury site and shorten the treatment period. Recently, improvements in the properties of the skin transplantation site and shortening of the treatment period have been reported

in many patients by applying Fiblast<sup>®</sup> Spray (Kaken Pharmaceutical Co., Ltd.) to the graft surface (2-5). However, it has also been recognized that hypertrophic cicatrix or keloid is likely to be suppressed at a mild level even in deep burns by appropriate treatment from an early stage (6).

We have performed some studies on the characteristics of ERI (7-9). ERI is water containing a large amount of electrons through the electrolysis of natural water, followed by electric current/pressure application using a special diaphragm system. ERI shows cleansing, deodorizing, antimicrobial, and anti-dust effects because dirt and bacteria, as the causes of odor, are detached and removed by its specific alkaline property and negatively charged ions (7). This water also has rust-preventing and anti-septic effects. In addition, stable emulsions could be prepared by the

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emulsification of various types of oil using ERI alone without emulsifiers, showing its emulsifying effect (8). Taking advantage of these properties, ERI is widely used at present as a cleansing agent incorporated in various industrial products.

We previously prepared magnesium aluminum silicate (Smectone<sup>®</sup>) gels using ERI as a dispersal medium for medical drugs, and evaluated their physicochemical properties (10,11). As a result, the use of ERI, compared with purified water, facilitated the preparation of drug delivery system (DDS) drugs with the maintenance of the gel state. These results showed that the use of gels with functions maintained using the specific properties of ERI is useful for preparing percutaneously absorbed drugs such as sustained-release preparations.

This report presents a girl with a third-degree burn that could be treated satisfactorily by conservative therapy using ERI lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment without dermatoplasty, which was rejected by her parents.

## 2. Materials and Methods

### 2.1. Materials

As ERI, S-100<sup>®</sup> (A. I. System Product Co., Japan) was used. ERI lotion containing 2% vitamin C was from A. I. System Product Co., Japan. For the antibiotics/steroid combination ointment, Hysetin-P<sup>®</sup> (chloramphenicol, fradiomycin sulfate, and prednisolone combination) was from Fuji Pharmaceutical Industry Co., Ltd., Japan. The vitamin A/E ointment, Juvela<sup>®</sup> (vitamin A and tocopherol combination), was from Eisai Co., Ltd., Japan. All reagents were of special grade.

### 2.2. Methods

The burn was treated by the application of ERI lotion, antibiotics/steroid combination ointment (Hysetin-P<sup>®</sup>), and vitamin A/E ointment (Juvela<sup>®</sup>) to the injury site 3 times a day combined with wrap therapy, *i.e.*, wrapping the injury site with a thin plastic film to prevent drying (12).

## 3. Results and Discussion

The patient was a 2-year-and-4-month-old girl. After she suffered a burn to the neck due to boiling water, she was examined at another hospital, was recommended to undergo dermatoplasty, and consulted our hospital 13 days after injury. A wide skin defect was observed around the right side of the neck, and a third-degree burn was diagnosed.

Because of the strong wish of her parents to avoid dermatoplasty, treatment was started by the application of ERI lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment to the wound 3 times a day

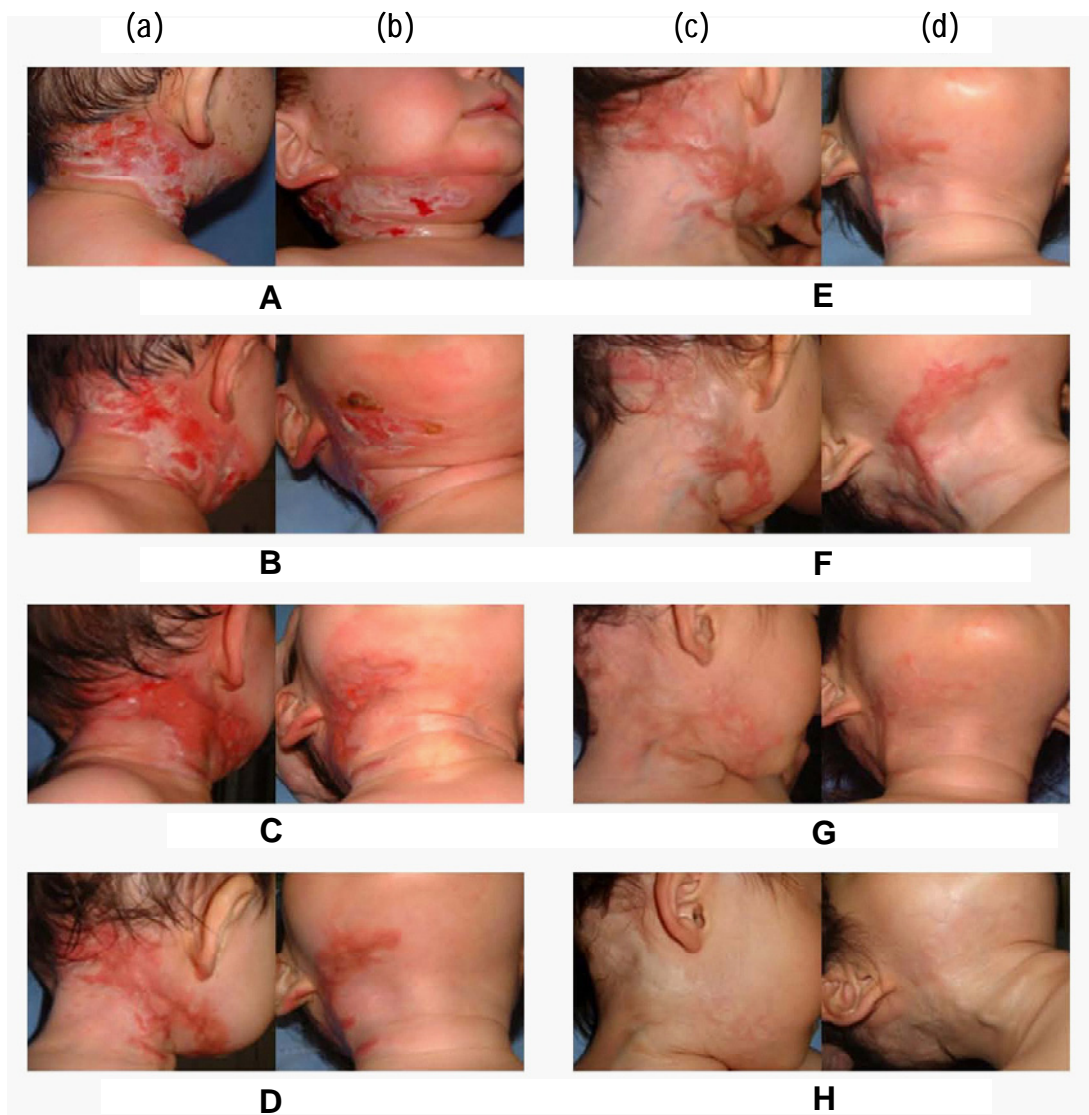
combined with wrap therapy. Figure 1A shows the state of the third-degree burn soon after the beginning of treatment. Figure 1B shows the state after 1 week, when the lysis of necrotic tissue and granulation formation started. Two weeks after the beginning of treatment, the necrosed skin had completely lysed, satisfactory granulation tissue formation was observed, and the blood supply improved. After 3 weeks, the concavity gradually began to flatten. Figures 1C-1E show the states 1-3 months after the beginning of treatment. Skin regeneration was noted, epithelialization progressed, and the area of the skin defect began to decrease. Figure 1F shows the state after 4 months, when epithelialization had completed, but hypertrophic cicatrix and pigmentation began to appear. After 6-9 months, the area of hypertrophic cicatrix gradually decreased, being replaced by normal skin. Figure 1G shows the state after 13 months. Capillary vessel proliferation was noted, cicatrix became inconspicuous, and pigmentation was resolved, showing satisfactory healing. Figure 1H shows the state after 26 months. Normal skin was restored in the area of cicatrix, and no functional disorder remained.

Usually, the treatment of burns often requires surgical skin grafting depending on the depth of the wound in addition to conventional external drug treatment. In the present patient, to comply with the strong wish of her parents for outpatient treatment without skin grafting, we performed conservative treatment using ERI lotion containing vitamin C, antibiotics/steroid combination ointment, and vitamin A/E ointment. Vitamin C was added to the ERI lotion in the process of its manufacturing, because vitamin C has been reported to promote wound healing (13). In this patient, the processes of tissue repair in the healing of the burn, *i.e.*, inflammation, granulation, and reconstruction, progressed smoothly, and cure could be achieved without leaving hypertrophic cicatrix, keloid scar, or pigmentation. This case is considered to demonstrate that third-degree burns can be treated simply by external treatment using ERI lotion, antibiotics/steroid combination ointment, and vitamin A/E ointment.

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**Figure 1.** A case of treatment of a burn wound of the right neck region with ERI lotion, antibiotic/steroid combination ointment, and vitamin A/E ointment employing wrap therapy. (a) The rear right side of the neck region, (b) the front right side of the neck region. The photographs (c) and (d) are from the same patient. (A) Before treatment. (B) 1 week after treatment. (C) 1 month after treatment. (D) 2 months after treatment. (E) 3 months after treatment. (F) 4 months after treatment. (G) 15 months after treatment. (H) 26 months after treatment.

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**Original Article**

## A study of the relationship between mental health and menstrual abnormalities in female middle school students from post-earthquake Wenchuan

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**Summary**

The present field investigation sought to explore the relationship between mental health and abnormal menstruation in female middle school students from post-earthquake Wenchuan following the 2008 Sichuan earthquake (the earthquake's epicenter was in Wenchuan County, Sichuan Province). A total of 587 female middle students from post-earthquake Wenchuan were given the PCL-C, SCL-90, and a menstruation questionnaire. Outcomes were measured by diagnostic criteria. The general incidence of PTSD was 60.8%, and D symptoms were the most prevalent PTSD symptoms (49.6%). Of symptoms indicated by the Symptom Checklist, obsessive-compulsive traits were most prevalent (94.6%), followed by interpersonal sensitivity (91.9%). The incidence of abnormal menstruation was 76.6%. Incidence of abnormal menses among students who screened positive for PTSD was significantly higher than among students who did not ( $\chi^2 = 4.015, p = 0.045$ ). The incidence of abnormal menses was higher among students who screened positive for somatization, obsessive-compulsive traits, phobic anxiety, and diet and sleep disorders than among those who did not ( $p < 0.05$ ). In conclusion, there was a relationship between mental health and physical health in female middle school students in a post-earthquake area. A higher incidence of abnormal menstruation may occur in students with PTSD, somatization disorder, obsessive-compulsive disorder, phobic anxiety, and diet and sleep disorders. Therefore, psychological intervention is particularly necessary for female students who have survived a natural disaster like an earthquake.

**Keywords:** Earthquake, abnormal menstruation, post-traumatic stress disorder (PTSD), mental health, female middle school student, relationship

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### 1. Introduction

The 8.0 degree earthquake that occurred in Wenchuan County and surrounding areas of Sichuan Province on May 12th, 2008 resulted in an enormous loss of life and property for residents and also seriously affected the physical and mental health of survivors. History has shown that a massive disaster will result in varying

degrees of impacts to people's mental health (1). Posttraumatic stress disorder (PTSD) is an anxiety disorder that occurs in the aftermath of a traumatic event (2). Examples are combat, rape and natural disasters. There are three major types of PTSD symptoms. First, the traumatized person generally develops a heightened startle response and easy arousability and irritability. Second, they are vulnerable to having memories of the trauma come flooding back into their minds at unexpected moments (flashbacks). Third, they will go to great lengths to avoid thinking about the trauma. These avoidance measures vary from not going near anything that reminds them of the trauma to dissociation. PTSD is the most common psychopathological symptom after

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disaster and may seriously affect the regular lives of survivors (3). PTSD can also cause survivors various psychological problems such as fear, helplessness, horror, depression, anxiety, hostility, paranoia, and diet and sleep disorders accompanying the threat of injury and death (4,5). An earthquake, as a strong stress factor, can lead to functional disorders by affecting the pituitary *via* the cerebral cortex and hypothalamus. Adolescent female students are in a period of dramatic physiological and psychological changes and may have emotional changes, feel powerless, have premature mental processes, and lack the ability to deal with stress. They may thus be more prone to develop psychological disorders, subsequently leading to physiological abnormalities. This study sought to ascertain how mental health affects the physical health of female students by analyzing psychological stress, mental health disorders, and menstrual abnormalities and their relationship to psychological states.

## 2. Methods

### 2.1. Subjects

A cluster sampling method was used to select 587 female middle school students in grades seven and eight who transferred from Wenchuan to Chengdu to continue their studies. This study took place 9 months after the Wenchuan earthquake on May 12, 2008.

### 2.2. Instruments of the survey

#### 2.2.1. Questionnaire on general condition

To collect personal data and ascertain the general status of subjects, subjects were given a questionnaire asking about their sex, age, grade, ethnicity, residence before the earthquake, injuries, and property damaged by the earthquake.

#### 2.2.2. Questionnaire on general condition

Symptoms of PTSD were evaluated using the PTSD Checklist-Civilian Version (PCL-C) including 3 symptoms and a total of 17 items. B symptoms of intrusion were indicated by 5 items, C symptoms of avoidance were indicated by 7 items, and D symptoms of high-alertness were indicated by 5 items.

#### 2.2.3. NOSIMH mental health self-rating scale (Symptom Checklist 90, SCL-90)

Symptom Checklist 90 (SCL-90) is an instrument to assess mental health and consists of 90 items concerning aspects like emotions, thoughts, consciousness, behavior, living habits, interpersonal relationships, diet, and sleep.

### 2.2.4. Menstruation questionnaire

To collect menstruation-related information on subjects, students were given a menstruation questionnaire asking their age of menarche and whether they had signs of abnormal menstruation (epimenorrhea, oligomenorrhea, irregular menstruation, hypermenorrhea, amenorrhea, and dysmenorrhea).

### 2.3. Survey methods

A survey of female middle students was conducted by trained research assistants (with onsite supervision by psychologists) with a pre-structured questionnaire. The questionnaires were self-administered to classes as a whole and collected on the spot.

### 2.4. Diagnostic criteria

#### 2.4.1. PTSD diagnostic criteria according to the PCL-C

According to the U.S. Diagnostic and Statistical Manual of Mental Disorders: 4th Edition (DSM-IV) (6-8), the PCL-C contains 17 yes/no items, with answers on a 4-point scale from 1 ("mild") to 2 ("moderate"), 3 ("severe"), and 4 ("very severe"). An "asymptomatic" answer was given a score of 0. A total PTSD symptom score was obtained by tallying all items. A higher total score suggests a greater possibility of PTSD occurring. A score sheet provides instructions for tabulation of the total score, and B, C, and D symptom subscale scores. An individual with a score higher than 38 was classified as screening positive for PTSD. If the individual has a score  $\geq 2$  points for one or more items in the five items for group B symptoms, she is classified as screening positive for group B symptoms. If the individual has a score  $\geq 2$  points for three or more items in the seven items for group C symptoms, she is classified as screening positive for group C symptoms. If the individual has a score  $\geq 2$  points for two or more items in the five items for group D symptoms, she is classified as screening positive for group D symptoms.

#### 2.4.2. SCL-90 diagnostic criteria (9,10)

The Symptom Checklist-90 (SCL-90) is a 90-item self-report symptom inventory with ten scales for symptoms (F1-F10) including somatization, obsessive-compulsive traits, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism, and diet and sleep. Each symptom scale consisted of several items. The questionnaire contains 90 yes/no items with a 5-point scale to measure responses ranging from 1 ("asymptomatic") to 5 ("very severe").

### 2.4.3. Abnormal menstruation (11)

An individual who comply with any one of the following symptoms, can be sentenced for abnormal menstruation: *i*) Epimenorrhea: Menstrual cycle shorter than 24 days. *ii*) Oligomenorrhea: Menstrual cycle longer than 35 days. *iii*) Irregular menstruation: Irregular menstrual cycle, heavy/little menstruation or longer menstrual period ( $\geq 7$  days). *iv*) Hypermenorrhea: Regular metrorrhagia with heavy menstruation or long menstrual period (amount of bleeding  $\geq 80$  mL). *v*) Amenorrhea: Cessation of menstruation for 6 months or more than 3 months after the original self-menstrual cycle. *vi*) Dysmenorrhea.

### 2.4.4. Data analysis

The initial data was input using Epi Data 3.0 and the prevalence rates of symptoms of PTSD and SCL-90 and other characteristics were calculated and analyzed using SPSS version 13.0. Comparison of the rate used Chi-square test with the inspection level  $\alpha = 0.05$ .

## 3. Results

### 3.1. Demographic characteristics

A total of 587 middle students were surveyed; 586 questionnaires were collected with 569 valid responses. The valid response rate was 97.1%. Of 569 students participating in the survey, 246 (43.2%) were in grade seven and the remaining 323 (56.8%) were in grade eight. Two hundred and sixty-one (45.9%) were living in town before the earthquake and 308 (54.1%) were living in the country. Two hundred and ninety-one students (51.1%) were of the Qiang ethnic minority, 178 (31.3%) were of the Tibetan ethnic minority, 62 (10.9%) were of Han ethnicity, and 38 (6.7%) were of another ethnic minority. Of the students, 25 (4.4%) who were injured and 404 (71.0%) students suffered serious loss in household property in the earthquake.

### 3.2. PTSD screening results

Results of screening students for PTSD indicated that 346 (60.8%) were positive for symptoms, and the prevalence of D symptoms of PTSD was significantly higher among the students (49.6%) (Table 1).

### 3.3. Mental health

Of 569 students participating in the survey, their maximum score on the SCL-90 was 450, their minimum was 90, and the median was 141. The SCL-90 indicated that the most prevalent symptoms were obsessive-compulsive traits, followed by interpersonal sensitivity (Table 2).

**Table 1. Results of screening 569 female students for PTSD**

Group of symptoms	Total	Positive students	Prevalence (%)
B group (re-experience)	569	255	44.8
C group (avoidance)	569	106	18.6
D group (high-alertness)	569	282	49.6

**Table 2. Results of mental health screening of 569 female students with the SCL-90**

SCL-90 symptom scales	Total	Positive students	Prevalence (%)
Somatization	569	429	75.4
Obsessive-compulsive	569	538	94.6
Interpersonal sensitivity	569	522	91.9
Depression	569	507	89.3
Anxiety	569	490	86.1
Hostility	569	462	81.2
Phobic anxiety	569	488	85.8
Paranoid ideation	569	457	80.3
Psychoticism	569	480	84.4
Diet and sleep	569	496	87.2

### 3.4. Abnormal menstruation in female middle school students

There were 436 students who had a problem for abnormal menses among the 569 students participating in the survey (the incidence of abnormal menstruation was 76.6%). The incidences of abnormal menstruation among different grade, nationality, habitual residence before the earthquake, injuries and damage of property in earthquake were not statistically significant ( $p > 0.05$ ).

### 3.5. Relationship between PTSD and abnormal menstruation

The prevalence of abnormal menses among students who screened positive for PTSD and those who did not was 79.5% (275) and 72.2% (161), respectively ( $\chi^2 = 4.015$ ,  $p = 0.045$ ). The difference in the prevalence of abnormal menses among students who screened positive for B, C, and D symptoms and those who did not was not statistically significant ( $p > 0.05$ ) (Table 3).

### 3.6. Relationship between mental health and abnormal menstruation

Among the 569 students participating in the survey, the prevalence of abnormal menses was higher among students who screened positive for somatization, obsessive-compulsive traits, phobic anxiety, and diet and sleep disorders than among those who did not ( $p_{\text{somatization}} < 0.05$ ,  $p_{\text{obsessive-compulsive}} = 0.038$ ,  $p_{\text{anxiety}} = 0.002$ ,  $p_{\text{diet and sleep}} = 0.019$ ) (Table 4).

## 4. Discussion

Health is not only the absence of disease and illness but is also a comprehensive state of physical, mental, and



**Table 3. Distribution of PTSD and abnormal menstruation in 569 female students**

Variable	Result	Students	Cases of abnormal menses (%)	$\chi^2$	<i>p</i>
PTSD	negative	223	161 (72.2)	4.015	0.045
	positive	346	275 (79.5)		
Group of symptoms PTSD-B	negative	314	233 (74.2)	2.294	0.130
	positive	255	203 (79.6)		
PTSD-C	negative	463	351 (75.8)	0.923	0.337
	positive	106	85 (80.2)		
PTSD-D	negative	287	211 (73.5)	3.120	0.077
	positive	282	225 (79.8)		

**Table 4. Distribution of mental health disorders and abnormal menstruation in 569 female students**

Variable	Result	Students	Cases of abnormal menses (%)	$\chi^2$	<i>p</i>
Somatization	negative	140	88 (62.9)	19.654	< 0.05
	positive	429	348 (81.1)		
Obsessive-compulsive	negative	31	19 (61.3)	4.305	0.038
	positive	538	417 (77.5)		
Interpersonalsensitivity	negative	47	34 (72.3)	0.525	0.469
	positive	522	402 (77.0)		
Depression	negative	62	42 (67.7)	3.066	0.080
	positive	507	394 (77.7)		
Anxiety	negative	79	57 (72.2)	1.025	0.311
	positive	490	379 (77.3)		
Hostility	negative	107	76 (71.0)	2.305	0.129
	positive	462	360 (77.9)		
Phobic anxiety	negative	81	51 (63.0)	9.843	0.002
	positive	488	385 (78.9)		
Paranoid ideation	negative	112	81 (72.3)	1.442	0.230
	positive	457	355 (77.7)		
Psychoticism	negative	89	64 (71.9)	1.310	0.252
	positive	480	372 (77.5)		
Diet and sleep	negative	73	48 (65.8)	5.527	0.019
	positive	496	388 (78.2)		

social adaptability. Since the concept of stress was put forward by Selye, the relationship between psychosocial factors as a stressor and physical and mental health has garnered widespread attention (12). Cannon WB (13) found that the state of emotion significantly affects the physiological processes of individuals. He also pointed out that release of the hormone endocrine can be affected by intense changes in emotion *via* the nervous system by way of the hypothalamus. As a result, the cardiovascular system will be affected. This tends to lead to physiological dysfunction and eventually worsens into a pathological change if the harmful emotion is repeated experienced.

This survey used a PTSD self-rating scale (PCL-C) and self-reported symptoms checklist (SLC-90) to evaluate the mental health of female students from an area stricken by the Wenchuan earthquake. Results indicated that the prevalence of PTSD was 60.8% and

that the prevalence of each symptom on the SLC-90 was between 75.4%-94.6%. The students suffered a significant psychological trauma, especially in areas such as re-living the event, avoidance, high-alertness, somatization, obsessive-compulsive traits, phobic anxiety, sleep, and diet, since they have experienced the process of evacuation and awaiting rescue, some have witnessed the deaths of loved ones, and many lost their homes in the earthquake. A report by World Health Organization noted that victims of disasters have a serious physical and psychological reaction to the event (14).

A study by Hu and Liang found that the prevalence of PTSD was about 7.8%-80.0% in individuals who suffered severe trauma (15). Another study of the survivors of the 6.8 earthquake in Marathwada, India on September 30th, 1993 noted that 59% had problems like mental disorders (16).

The current study found that the prevalence of abnormal menses was higher among students who screened positive for PTSD and somatization, obsessive-compulsive traits, phobic anxiety, and diet and sleep disorders than among those who did not. This suggests that a strong stress response may lead to impaired regulation of psychological processes and the nervous and endocrine systems or of psychological processes and the nervous and immune systems. This thus leads to abnormal menstruation according to theories that blame activation of the hypothalamus-pituitary-adrenal axis (HPA), consistently increasing serum cortisol, consistently decreasing growth and sex hormones, impaired ovulation, or a poor response to estrogen and progesterone (17).

## 5. Conclusion

One of the most significant causes of an endocrine disorder in female students is a strong stress response. Some physical problems caused by psychological problems may affect adolescent female students' lives for a long time and may even become incurable. Post-disaster psychological intervention has become an essential part of disaster relief work in many countries. Therefore, in light of the physical and psychological problems noted after the Wenchuan earthquake positive intervention should be considered in order to relieve PTSD and other physical/psychological problems, helping victims recover psychologically and physically and restoring their mental health (18).

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**Original Article****Rapid increase in Japanese life expectancy after World War II****Yasuo Sugiura<sup>1,\*</sup>, Young-Su Ju<sup>2</sup>, Junko Yasuoka<sup>3</sup>, Masamine Jimba<sup>3</sup>**<sup>1</sup> Bureau of International Cooperation, International Medical Center of Japan, Ministry of Health, Labour and Welfare, Tokyo, Japan;<sup>2</sup> Department of Occupational & Environmental Medicine, Hallym University College of Medicine, Gyeonggi, Korea;<sup>3</sup> Department of Community and Global Health, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.

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**Summary**

Japanese life expectancy increased by about 13.7 years during the first decade after World War II, despite the country's post-war poverty. Although it is known that medical progress explains part of this increase, roles of non-medical factors have not been systematically studied. This study hypothesizes that non-medical factors, in addition to medical factors, are associated with the rapid increase in life expectancy in Japan. We analyzed the time trends of potential explanatory factors and used regression analysis with historical data from the Ministry of Internal Affairs and Communications' Historical Statistics of Japan during the period between 1946 and 1983. Time trends analysis revealed that the rapid increase in life expectancy preceded the dramatic growth of per capita Gross Domestic Product (GDP) by 10 years. In education, the nearly universal enrollment in elementary schools and increased advancement to upper secondary schools for both sexes were associated with better health. Regarding legislation, 32 health laws were passed in the first decade after the war and these laws were associated with improved health. Using regression analysis, we found that the enrollment rate in elementary schools, the number of health laws, and expansion of community-based activity staff were significantly associated with the increased life expectancy during the first decade after World War II. To conclude, in addition to medical factors, non-medical factors applied across the country, particularly education, community-based activities and legislation were associated with the rapid increase in Japanese life expectancy after World War II.

**Keywords:** Japanese life expectancy, per capita GDP, education, law, community-based activity

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**1. Introduction**

The Japanese currently have the longest life expectancy at birth in the world, averaging 83.0 years among men and women (1). Numerous factors have been associated with the high life expectancy in Japan, such as high quality of health services, a national health insurance system, a social security system, economic prosperity, as well as nutritional and environmental factors (2-4).

The decade following World War II was a breakthrough

period, during which Japanese life expectancy dramatically increased by an average of 13.7 years: 13.5 years for males (from 50.1 to 63.6 years) and 13.8 years for females (from 54.0 to 67.8 years) between 1947 and 1955. Factors used to explain this phenomenon include: rigorous public health campaigns aimed at controlling infectious diseases, the widespread use of antibiotics and chemotherapeutic drugs in medical practice, and urbanization (5-7). Epidemiological research indicates that improved management of gastroenteritis, pneumonia and tuberculosis significantly contributed to the survival rate of children and youth in the 1950s and the 1960s (8). Since life expectancy is calculated by a life table that contains the proportion of people dying at each age interval, decline in infant mortality and mortality caused by major infectious diseases would have had considerable impact on life expectancy.

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While these explanations for increased life expectancy are plausible, little systematic analysis has been conducted on the societal changes that occurred in the 10 years following the war. In countries such as the United Kingdom (England and Wales), the United States, and India, life expectancy has gradually increased since the end of the war (9). Further, there is evidence of positive correlation between national income and life expectancy worldwide (10), and it is generally believed that the Japanese economic growth contributed to raising Japanese life expectancy after World War II. However, we wondered whether economic growth would sufficiently account for the rapid increase in life expectancy during the decade after the war in Japan. The Japanese economy on the whole was weakened by war, and it took nearly 10 years to restore it to pre-war levels through substantial financial support from the United States. Further, people faced serious food shortages due to the poor rice crop in 1945, exacerbated by an inefficient system of food distribution. This study hypothesizes that non-medical factors, in addition to medical factors, were associated with the rapid increase in life expectancy in Japan. Determinants of mortality have been argued to include nutrition, hygienic measures, economic development, literacy, and health-related knowledge and technology (11-14). In this study, we focused on the state of the economy, nutrition, and education as basic background variables of health outcomes and examined legislation and community-based activities to reflect top-down and bottom-up health initiatives, respectively.

We begin with an examination of the relationship between Japanese economic growth and life expectancy. Then, we analyzed non-medical factors to examine how these factors are associated with the rapid increase in Japanese life expectancy.

## 2. Methods

We applied a time trend analysis between 1946 and 1983 using annual data from different governmental organizations to examine potential explanatory factors for the rapid increase in Japanese life expectancy. The definition of life expectancy in this article is average number of years that a newborn is expected to live if current mortality rate continues to apply. Life expectancy is calculated by a life table that contains the proportion of people dying at each age interval.

### 2.1. Data collection

Using the Ministry of Internal Affairs and Communications' Historical Statistics of Japan, annual data were collected on life expectancy, population, daily intake of energy and protein, height, the number of public health centers and public health nurses; enrollment rates in elementary schools and advancement

rate to upper secondary schools; death rate by pneumonia and tuberculosis; infant mortality rate; and annual average of monthly household disbursements. Gross Domestic Product (GDP) data were obtained from the Bank of Japan and the Organization for Economic Cooperation and Development (15). Legislative data were obtained from the Law and Ordinance Data Service System (16). The numbers of community-based activity staff, who were called Livelihood Extension Workers, and their activities for rural life improvement were obtained from the Annual Report of the Cooperative Agricultural Extension Service in the Ministry of Agriculture and Forestry. Engel's coefficient is a proportion of family income that is spent on food. The lower the family's income, the greater the proportion of the income that is spent on food (17). In general, high Engel's coefficient indicates poor and low Engel's coefficient relates rich.

### 2.2. Data analysis

All raw data were presented in the form of time-series figures to show their relationships. Regression analyses were conducted by using the differences of two continuous years for all variables from 1948 to 1983, except for the data on the rate of advancement to upper secondary schools from 1950 to 1983. A time factor was used in the multivariate model for correcting for the time effect. We also considered multicollinearity effects by examining the correlation coefficients and standard errors among the explanatory variables. As for non-medical factors in the regression analyses, we added the average height of a 15-year-old female as an indicator of nutritional conditions. As for medical factors, we selected death rates from tuberculosis and pneumonia, which were the leading causes of death in Japan from 1899 to 1950, and the infant mortality rate as one of the main factors affecting life expectancy. In addition, we added the number of health centers and public health nurses in health centers as indicators of public health measures. All statistical analyses were performed by the statistical package, SAS 8.1 version.

## 3. Results

### 3.1. Association between Japanese life expectancy and per capita GDP

After World War II, both Japanese life expectancy and per capita GDP increased dramatically. However, there is a decade of time lag between these changes (Figure 1). After the war, it took about 10 years for the per capita GDP to rebound to the prewar level. Despite the delay, during that time, the Japanese life expectancy rapidly increased from 50.1 to 63.6 years for males and from 54.0 to 67.8 years for females. After 1955, the per capita GDP sharply increased until 1972, which was

considered to be the period of most rapid economic growth in Japan. Hence, the rapid increase in Japanese life expectancy preceded the rapid economic growth.

3.2. Daily intake of energy and protein in Japan after World War II

We divided the period from 1946 to 1983 into three phases based on the daily intake of energy and protein after World War II: a restorative phase, a stable phase, and a variable phase (Figure 2). In the restorative phase, Japan experienced serious food shortages and received

food aid from General Headquarters, the Supreme Commander for the Allied Powers (GHQ/SCAP), UNICEF and NGOs such as, Licensed Agencies for Relief in Asia (LARA) and Cooperative for American Remissions to Europe (CARE). During this phase, the average Japanese daily intake of energy and protein rapidly increased from 1,903 kcal and 59.2 g in 1946 to 2,125 kcal and 68.3 g in 1951, respectively. In the stable phase between 1952 and 1963, both the energy and protein intake remained at approximately 2,100 kcal and 70 g, which was considered to be sufficient nutritional intake at that time. In the variable phase after 1964,

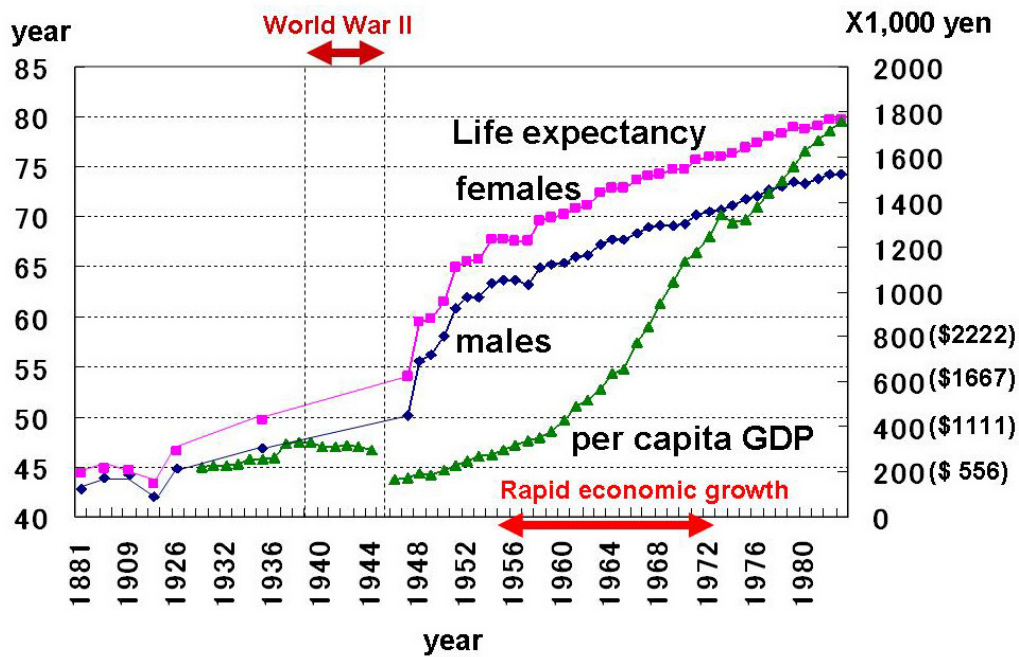


Figure 1. Life expectancy and GDP/capita in Japan. The US dollar figures shown in the parentheses at the right vertical scale were converted from the Japanese yen amounts at the rate of 360 yen/dollar.

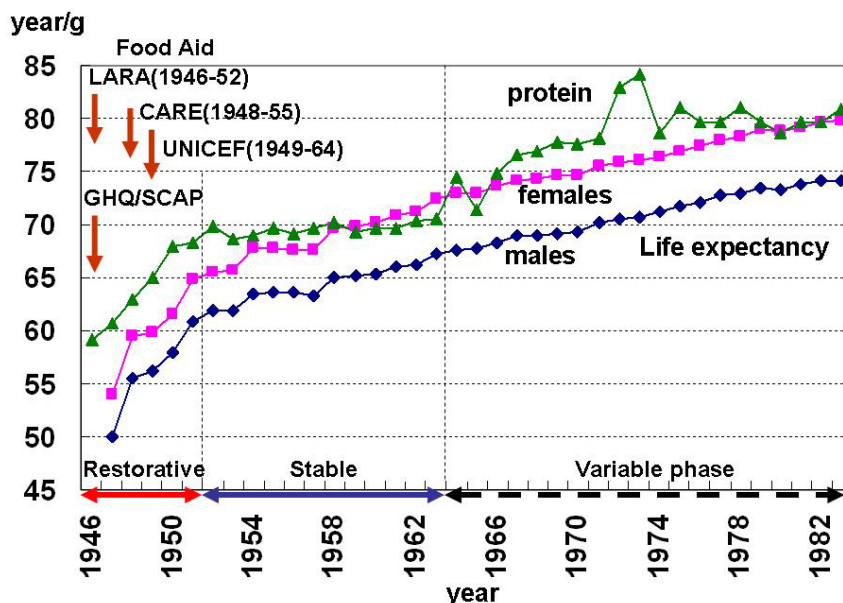


Figure 2. Life expectancy and daily intake of protein in Japan.

however, daily intake of energy and protein varied due to changing eating habits and life style.

### 3.3. Compulsory elementary and upper secondary education and health in Japan

Despite upheaval in education policy and practice brought about by the GHQ/SCAP, the rate of enrollment in elementary schools was consistently above 99.6% during the first decade after World War II (Figure 3). Following GHQ/SCAP recommendations, nationwide school lunch programs began in 1946, resulting in increased body weight and height of school children. This led the people and the government to enact the school lunch law in 1954. Based on the principal of equal opportunity for the education of both sexes, the rate of advancement to upper secondary schools was 55.5% for boys and 47.4% for girls by 1955. By 1975, 99.9% of young Japanese children were enrolled in elementary schools, and by 1973, 88.3% of males and

90.6% of females advanced to upper secondary schools. The school health law of 1958 introduced required regular physical checkups and improvement in school hygiene and environment. Health education was also included as a regular subject in the curriculums at the upper secondary schools.

### 3.4. Health laws in Japan

Fifty-five health laws went into effect between 1946 and 1983 in Japan. Most of the health laws (32 out of the 55) were enacted between 1946 and 1955. Additional health laws were gradually introduced between 1956 and 1983. We categorized 55 health laws into 10 groups over the four decades (Table 1). Ten out of 32 health laws enacted between 1946 and 1955 were classified as human resource qualification laws, which regulates licenses to medical doctors, public health nurses, midwives, radiological technologists, and nutritionists, among others. In total, 20 out of the 55

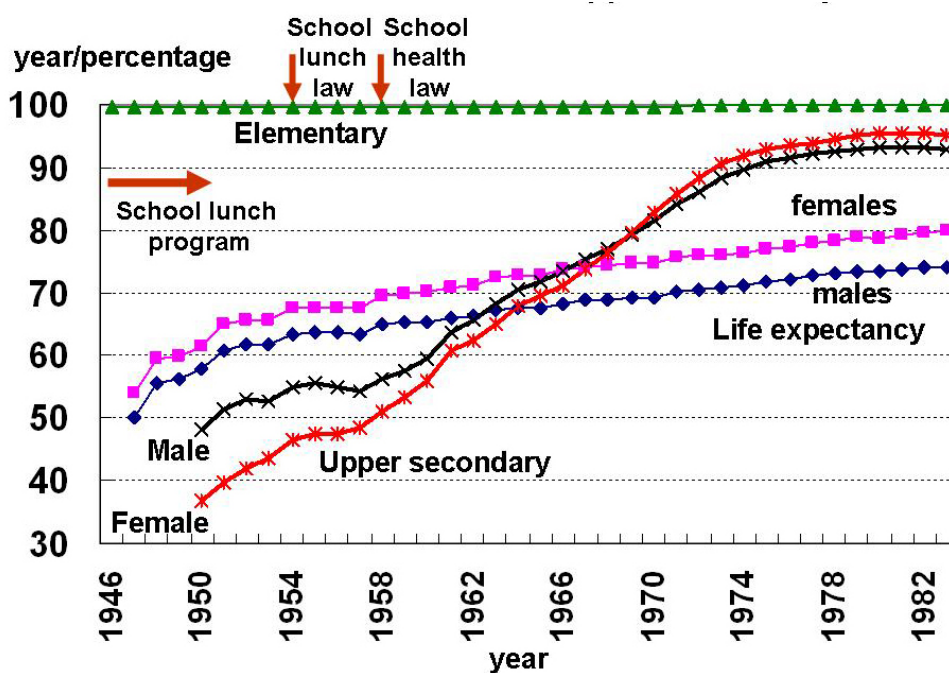


Figure 3. Life expectancy and enrollment rate in elementary schools and advancement rate to upper secondary schools.

Table 1. Number of new health laws introduced in each time period

	1946-1955	1956-1965	1966-1975	1976-1983	Total
Human resource qualification	10	5	5	0	20
Environmental health	6	2	2	3	13
Disease prevention	5	0	0	0	5
Drug control	5	0	0	0	5
Medical services	3	2	0	0	5
Public health center	1	1	0	0	2
Education	1	0	0	1	2
Food sanitation	1	0	0	0	1
Management	0	1	0	0	1
Biological weapon	0	0	0	1	1
Total	32	11	7	5	55



health laws between 1946 and 1983 were classified as the human resource qualification laws.

3.5. Community-based activity staff and their activities

After World War II, drastic agricultural land reform was implemented in 1946 and contributed to equality among farm households in Japan. Community-based activity staffs, known as Livelihood Extension Workers, were introduced by the Agricultural Improvement Promotion law of 1948. The Livelihood Extension Workers were women who paid frequent visits to villages to listen carefully to problems in the farmers' daily lives. They served as important facilitators who helped address many issues to improve the quality of life in rural areas. During the first decade after the war, the number of the Livelihood Extension Workers grew to 1,476. Their ranks were further increased to 2,350 by 1966. Thus, community-based activities to improve living standards in this rural society expanded nationwide after the war.

3.6. Regression analyses for life expectancy and possible determinants

To examine the associations between different medical and non-medical factors and the rapid increase in life expectancy after World War II, we applied univariate regression analysis using these factors. Among the non-medical factors, there were six significant variables: number of Livelihood Extension Workers ( $p = 0.001$ , Adjusted  $R^2 = 0.26$ ), enrollment rate in elementary schools ( $p = 0.002$ , Adjusted  $R^2 = 0.23$ ), rate of advancement to upper secondary schools ( $p = 0.006$ , Adjusted  $R^2 = 0.19$ ), accumulated number of health laws ( $p = 0.006$ , Adjusted  $R^2 = 0.19$ ), height in 15 year old females ( $p = 0.008$ , Adjusted  $R^2 = 0.17$ ), and Engel's coefficient ( $p = 0.045$ , Adjusted  $R^2 = 0.09$ ). Per capita GDP ( $p = 0.153$ ), daily intake of energy ( $p = 0.166$ ) and daily intake of protein ( $p = 0.373$ ) were not significantly associated with life expectancy. Among the medical factors, there were four significant variables: the number of public health centers ( $p < 0.001$ , Adjusted  $R^2 = 0.49$ ), pneumonia death rate ( $p < 0.001$ , Adjusted  $R^2 =$

0.45), tuberculosis death rate ( $p < 0.001$ , Adjusted  $R^2 = 0.43$ ) and infant mortality rate ( $p = 0.009$ , Adjusted  $R^2 = 0.16$ ). The number of public health nurses in health centers ( $p = 0.737$ ) was not significantly associated with life expectancy.

We then applied multivariate regression analysis to examine the influence of non-medical factors by adjusting medical factors (Table 2). Among the non-medical factors, four variables were significantly associated with life expectancy: enrollment rate in elementary schools ( $p < 0.001$ ), the number of Livelihood Extension Workers ( $p = 0.009$ ), accumulated number of health laws ( $p = 0.015$ ), and height in 15 year old females ( $p = 0.032$ ). The Engel's coefficient ( $p = 0.156$ ) and per capita GDP ( $p = 0.921$ ) were not significantly associated with life expectancy. Among the medical factors, two variables were significantly associated with life expectancy: pneumonia death rate ( $p < 0.001$ ) and the number of health centers ( $p = 0.047$ ). The adjusted R-square of the model was 0.84.

3.7. A relationship among medical factors, non-medical factors and Japanese life expectancy after World War II

Based on the results of our study, combined with previous studies, we diagrammed medical factors and non-medical factors that led to the rapid increase in Japanese life expectancy after World War II (Figure 4). We classified medical factors into three categories, including health outcomes, health technology, and health facilities and staff. We classified non-medical factors in two categories: individual and nationwide systems. Our study indicates both medical and non-medical factors were associated with increased life expectancy prior to the rapid economic growth that began about 10 years later.

4. Discussion

Our study demonstrates that non-medical factors such as education, health laws, and community-based activities were associated with the rapid increase in Japanese life expectancy after World War II. We

Table 2. Multivariate analysis of non-medical and medical factors on life expectancy (1948-1983)

	Factor	Parameter estimate	Standard Error	t Value	Pr >  t
Non Medical	Enrollment rate in elementary schools (%)*	14.673	3.909	3.75	< 0.001
	Number of Livelihood Extension Workers*	0.002	0.001	2.83	0.009
	Accumulated number of health laws*	0.125	0.048	2.61	0.015
	Height of 15 year old females (cm)*	0.633	0.279	2.27	0.032
	Engel's coefficient (%)	11.715	8.014	1.46	0.156
	per capita GDP (yen)	-0.0002	0.002	-0.10	0.921
Medical	Death rate from pneumonia (Number of pneumonia deaths per 100,000 population)*	-0.066	0.012	-5.44	< 0.001
	Number of health centers*	0.023	0.011	2.08	0.047

Adjusted R-square = 0.84; \* Statistically significant ( $p < 0.05$ ).

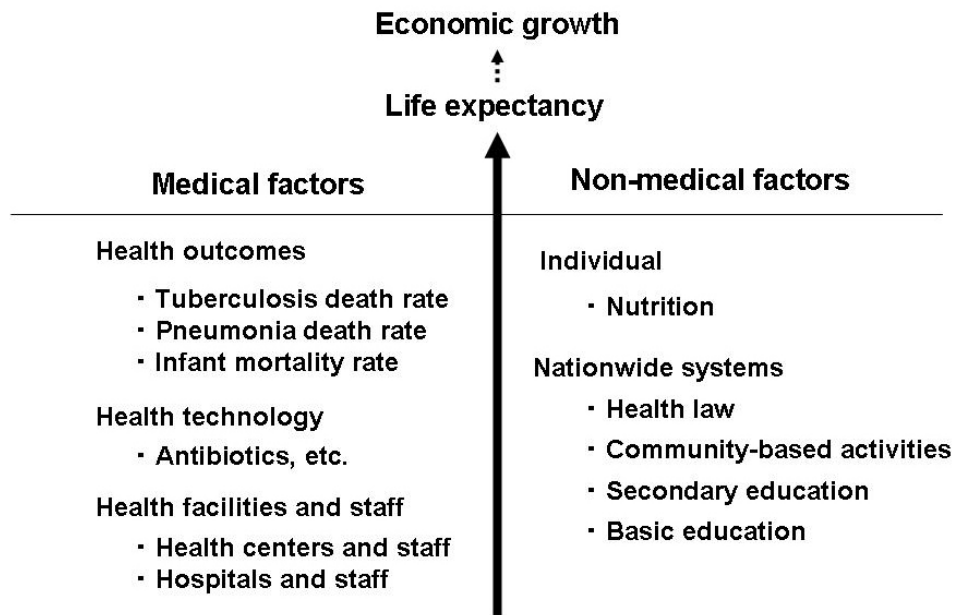


Figure 4. Medical and non-medical factors that led to rapid increase in Japanese life expectancy.

also found that the rapid increase in life expectancy preceded the rapid growth of the Japanese economy.

Our analysis of life expectancy and per capita GDP between 1946 and 1983 indicates that the rapid increase in Japanese life expectancy preceded the dramatic growth in per capita GDP by a decade. This runs counter to the analysis by Preston, who assessed the impact of economic conditions on mortality using data from the 1930s and 1960s, and concluded that in Japan, for example, a majority of the expected increase in life expectancy was caused by the rapid national income growth (12). We believe this inconsistent interpretation derived from differences between Preston's two decades analysis and our time trend analysis. Bloom *et al.* found that improved health contributes to rapid growth of per capita GDP, based on cross-country growth regressions (18), which is consistent with our results. Based on this theory, the time lag between the rapid increase in life expectancy and per capita GDP can be explained by the growing number of healthier people and their accumulated savings fueling the rapid growth of the Japanese economy from 1955 to 1972. Cutler *et al.* describe a similar scenario in China, where a reduction in infant mortality occurred prior to the acceleration in economic growth after 1980, which led them to downplay direct causal mechanisms running from income to health (14).

Regarding nutrition and life expectancy between 1946 and 1983, our results indicate that daily intake of energy and protein at the restorative phase from 1946 to 1951 may have affected the rapid increase in Japanese life expectancy. Body weight and height in school children decreased after the onset of World War II in 1941, and took about 10 to 20 years to rebound to their pre-war levels. These facts suggest that most Japanese

people were under calorie restriction for quite a long time. This is interesting to consider in light of research into the link between calorie restriction and extending life span (19,20).

In terms of legislation between 1946 and 1983, we focused on the reformed health system which involved the implementation of many new health laws, and found that most of these laws were passed in the first decade after World War II. The reason for the large numbers of new health laws is attributable to GHQ/SCAP occupation between 1945 and 1952 (21). Moreover, it is striking that the Japanese people followed the laws, even though they were devastated by the war. However, we believe that the important point is not the sheer number of health laws, rather political commitment to construct a health system and to train qualified human resources to serve as medical staff, including medical doctors, nurses, and midwives. We also recognize that each law has an individual purpose to improve health status in Japan. For example, there are two health laws which particularly strongly affected the Japanese health system and life expectancy after the war. First, the Public Health Center law of 1947 shaped the health system in Japan. Under GHQ/SCAP supervision, public health centers were reorganized and established to manage local public health administration with vital statistics and to provide public health services nationwide (22-24). Second, the Tuberculosis control law of 1951 provided physical checkups for about 24 million people per year, and about 12.5 billion Japanese yen (34.7 million US dollar) per year through government commitment by 1955 (5,25). Thus, the death rate for tuberculosis decreased from 187.2 to 52.3 per 100,000 people from 1947 to 1955.

Our study suggests that the enrollment rate in



elementary schools and advancement rate to upper secondary schools may be associated with the rapid increase in life expectancy after World War II. We interpret this effect as showing that not just schooling itself, but attendant factors such as an equal educational opportunity for all, school lunch programs and health education all contributed to this increase in life expectancy. This high enrollment rate in elementary schools required political commitment and a general appreciation of education. Japan has a more than 130-year history of compulsory education. The government embarked on the provision of a modern school system with the *Gakusei* (Education Law) in 1872. Elementary schools were standardized regardless of socioeconomic status or gender (26). The enrollment rate increased to 50.3% by 1891 and reached 98.1% by 1909 (27).

Caldwell pointed out that Japan and Sri Lanka had a similar experience of increases in life expectancy after World War II (28). Sri Lanka gained 12 years in life expectancy between 1946 and 1953. There were several similar factors in both countries: drastic advancement in the control of infectious diseases such as malaria in Sri Lanka and tuberculosis in Japan, improvement in the food supply, expansion of free education and school medical examinations, introduction of new therapeutic agents and technological innovation in curative and preventive medicine (29). However, the major difference is that Japanese society was deeply impacted by World War II. The war had caused people to rethink the meaning of a good life and a good society (30). The Japanese people strongly desired peace and wanted to share a happy family life. For instance, after the baby boom between 1947 and 1949, the people started to choose to have fewer children. With the enactment of the Eugenic Protection Law in 1948, which allows abortions for protection of motherhood, the total fertility rate fell dramatically from 4.57 in 1947 to 2.37 in 1955. The change seems to be associated with poverty reduction and health improvement in Japan.

From the experiences of Kerala in India, Sri Lanka and Costa Rica, Warren summarized four basic elements for good health at low cost; political and social will; education for all with emphasis on primary and secondary schooling; equitable distribution of health measures and primary health care; and assurance of adequate caloric intake for all (31). Our results indicate that Japan implemented these four elements after the war. Furthermore, Sen states that East Asian economies were comparatively early in their massive expansion of education, and later also of health care, before they broke the restraints of general poverty (32). Our results show that Japan was a good example of this phenomenon.

In this study, we found that Japanese life expectancy rapidly increased under the following conditions: 1) poor living standards before the Japanese economic

growth, 2) a restorative phase in calorie and protein intake, 3) very high enrollment rate in elementary schools and increasing advancement rate to upper secondary schools, 4) enactment of many health laws, and 5) increasing community-based activity staff and their activities. As one of the limitations of this study, we might have overlooked other factors which affect rapid increase in life expectancy. Further analysis needs to be done.

In conclusion, our study demonstrates that non-medical factors based on nationwide systems such as education, health laws, and community-based activities are associated with the rapid increase in Japanese life expectancy during the first decade after World War II. Health improvements preceded the rapid expansion of the Japanese economy. In this regards, post-war Japan exemplifies how compulsory education, political commitment and community initiative, rather than national income, improved health of the people. Lessons from Japan's experience demonstrate that the importance of deploying a multi-sector vision, within a nationwide system, and a strategy targeting long term impacts in a country by decision makers at various levels who are focused on vertical health issues.

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**Original Article****Bioavailability and biological activity of liquisolid compact formula of repaglinide and its effect on glucose tolerance in rabbits****Boushra M. El-Houssieny<sup>1</sup>, Lobna. F. Wahman<sup>2,\*</sup>, Nadia M. S. Arafa<sup>3</sup>**<sup>1</sup>Department of Pharmaceutics, National Organization for Drug Control and Research (NODCAR), Giza, Egypt;<sup>2</sup>Department of Biology and Hormonal Evaluation, National Organization for Drug Control and Research (NODCAR), Giza, Egypt;<sup>3</sup>Department of Physiology, National Organization for Drug Control and Research (NODCAR), Giza, Egypt.**Summary**

This study is an extension of the previous enhancement of dissolution properties of repaglinide using liquisolid compacts. The development and validation of a high-performance liquid chromatography (HPLC) assay for the determination of repaglinide concentration in rabbit plasma for pharmacokinetic studies is described. Repaglinide optimizing formula was orally administered to rabbits and blood samples were used to determine the pharmacokinetic parameters of repaglinide, which were compared to pharmacokinetic parameters of marketed tablets (Novonorm 2 mg). Also, to investigate the biological activity of this new formula, in comparison with the commercial product, oral glucose tolerance tests (OGTT), area under the curve and insulin levels were studied. Moreover, we studied the efficacy and safety of this new formula in several potencies (0.5, 1, and 2 mg) and blood glucose, insulin, kidney and liver functions. The relative bioavailability of repaglinide from its liquisolid compact formula was found to be increased significantly in comparison to that of the marketed tablet. In regard to urea and creatinine, no significant change was recorded after the administration of the commercial and the three potencies of the new formulation compared with the control group. Similarly, in liver function tests (serum glutamic pyruvic transaminase, SGPT), there were no changes observed in its level. Regarding insulin levels, the commercial formula increased insulin levels insignificantly (3.52% change) while the new formula increased the insulin level significantly with a percent change of 37.6%. The results of the glucose tolerance test showed that the blood glucose level was decreased significantly after the commercial drug (percent change, 18.1%) while in groups treated with the new formulation the decrease was highly significant ( $p < 0.01$ ) with a percent change of 29.98%. The change in area under the curve for blood glucose was significantly higher in the commercial drug plus glucose load than in the new formulation plus glucose load group ( $p < 0.05$ ) in the periods of 30-45 min and 45-60 min. Furthermore, the new repaglinide formulation significantly decreased blood glucose levels more than the commercial formula.

**Keywords:** Repaglinide, glucose tolerance, bioavailability, insulin, pharmacokinetics

**1. Introduction**

Type 2 diabetes mellitus is a complex heterogeneous metabolic disorder in which peripheral insulin resistance

and impaired insulin release are the main pathogenetic factors. The rapid response of pancreatic beta-cells to glucose is already markedly disturbed in the early stages of type 2 diabetes mellitus. The consequence is often postprandial hyperglycemia, which seems to be extremely important in the development of secondary complications, especially macrovascular disease. Meglitinide analogues are a class of oral hypoglycemic agents that increase insulin secretion, in particular, during the early phase of insulin release (1,2).

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The starting point for type 2 diabetes therapy is a change in lifestyle, especially diet. Unfortunately, dietary and lifestyle measures alone achieve adequate glycemic control in only a minority of patients. Thus, oral hypoglycemic drugs are routinely prescribed for type 2 diabetic patients. Some of these drugs, such as metformin and thiazolidenediones, primarily exert their effects on extra-pancreatic insulin-target tissues while others directly target the pancreatic  $\beta$ -cell and induce insulin secretion (3).

Repaglinide is a new carboxymethyl benzoic acid derivative, also known as 2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl] amino]-2-exoethyl] benzoic acid. It is a novel post prandial glucose regulator for the treatment of type 2 diabetes mellitus (4,5). It reduces fasting glucose concentrations in patients with type 2 diabetes mellitus. It helps to control blood sugar by stimulating release of insulin from the pancreatic  $\beta$ -cell by closure of  $K_{ATP}$  channels (6). Repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. It differs from other antidiabetic agents in its structure, binding profile, duration of action and mode of excretion (7).

Several analytical methods are available for the determination of repaglinide in biological fluids, including an HPLC method and liquid chromatography-tandem mass spectrometry (LC/MS/MS) (8-10). However, these methods are costly and require the availability of expensive equipment.

The present study aimed *first* to develop a simple, rapid, easy to handle, accurate and inexpensive method for the quantification of repaglinide in plasma with a low limit of quantification (< 10 ng/mL). Application of a modified HPLC method for investigation of its pharmacokinetic and a bioequivalence study of the commercially available product versus the new repaglinide liquisolid compact formula as representative of the most promising formula according to the previous study was investigated (11). *Second*, the study aimed to investigate the biological activity of this new repaglinide liquisolid compact formula in comparison, with the commercial ones. This included their effect on oral glucose tolerance tests (OGTT), area under the curve and insulin levels. The study also illustrated the efficacy and safety of this new formulation by estimating the effect of several potencies of this new formulation (0.5, 1, and 2 mg) on blood glucose, insulin, kidney, and liver function.

## 2. Materials and methods

### 2.1. Reagents

Repaglinide was provided by Amoun Pharma, Egypt. Indomethacin was purchased from Sigma-Aldrich, St. Louis, Mo, USA. Coarse granular microcrystalline cellulose (Avicel PH101) was from FMC, Philadelphia, PA, USA; polysorbate 80 and sodium starch glycolate

**Table 1. Composition of repaglinide liquisolid compact formulation per 100 tablets**

Ingredients	Dose (mg)		
	0.5	1	2
Drug (mg)	50	100	200
Liquid (mg)	100	200	400
Avicel PH101 (g)	0.526	1.05	2.11
Calcium silicate (g)	0.105	0.211	0.421
Explotab (mg)	39.1	78.2	156.3
Magnesium stearate (mg)	7.82	15.63	31.26
100 tablet (mg)	828	1657	3314

(explotab) were purchased from Sigma Chemical Co., USA; calcium silicate was from BDH Chemicals, Ltd., Poole, UK; acetonitrile HPLC grade was from Ramil Chemicals, Liece, UK; ammonium acetate, potassium dihydrogen phosphate, sodium hydroxide, magnesium stearate, and sodium citrate were from El-Nasr Pharmaceutical Co., Egypt; ethyl acetate, ethanol, and isoamyl alcohol were from Merck, Darmstadt, Germany.

### 2.2. Equipment

The rotary tablet machine M 912-512L using a 14-standard round flat-face punch was from Stokes, USA and the compression force was adjusted to obtain tablets with hardness up to 7 kg. Vortex-mixer was Heidolph Reax Top; Heidolph Instruments, Germany, and centrifuge was Universal 16, Hettich, Germany.

### 2.3. Preparation and mixing of the powders of repaglinide liquisolid compact (11)

The liquisolid compact of repaglinide containing polysorbate 80 as liquid medication, Avicel PH<sub>101</sub> as a carrier and calcium silicate as a coat at excipient ratio 5, showing the most promising liquisolid compact formula for enhancement of repaglinide dissolution rate, was prepared according to the previous study (11). Table 1 represents the composition of repaglinide liquisolid compact formulation per 100 tablets. The concentration of repaglinide in polysorbate 80 used as liquid medication was 50% (12-14).

The repaglinide dose (0.5, 1, or 2 mg) was manually mixed with polysorbate 80 in the required ratio using a porcelain mortar until a homogenous mixture was obtained. The carrier (Avicel PH<sub>101</sub>) was then added to absorb the liquid. The excess fluid was absorbed by the coating material (calcium silicate), that was added later. This order of mixing was proved by Sheth *et al.* (15) to produce the most optimal release rate. Explotab (disintegrant) and magnesium stearate (lubricant) were added to tablet preparations in concentrations of 5% and 1% w/w, respectively.

### 2.4. Oral absorption profile of the prepared repaglinide liquisolid compact tablet



#### 2.4.1. Study design

Studies were carried out to compare the oral absorption of repaglinide (2 mg) from tablets containing liquid compact formula (treatment A) to commercial tablets (treatment B) following administration of a single dose each using a randomized crossover design.

A total number of twelve white male rabbits (Boskat breed) weighing from 1.5-2 kg were used as a model animal for determining the bioavailability of repaglinide. They were divided into two groups of six rabbits each. Animals were obtained from the animal house of the National Organization for Drug Control and Research (NODCAR). Animals were kept individually in cages with wire-mesh bases constructed of galvanized steel for two weeks before treatment for acclimatization in a room with controlled lighting (14 h/day), constant temperature (16-20°C) and relative humidity (55-65%). Sick, injured and abnormal animals were eliminated. Rabbits were fed a commercial pellet diet and water *ad libitum*.

Ten tablets from each treatment (A and B) were well pulverized and suspended in 50 mL distilled water. Five mL from each treatment were given orally to each rabbit with oral cannula. Blood samples (2 mL) were collected prior to administration and after 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, and 6 h of drug administration. Blood was collected in heparinized tubes. Blood samples withdrawn as above were transferred to a series of graduated centrifuge tubes containing 0.4 mL of 2.5% w/v sodium citrate solution. Samples were centrifuged at 2,500 rpm for 5 min. Plasma was transferred into another set of sample tubes and frozen until assay. One undosed plasma sample was kept as a blank. Samples were filtered through a 0.25 µm membrane filter (Millipore). The repaglinide concentration in blood samples was analyzed using the modified HPLC method (8,16).

#### 2.4.2. Chromatographic conditions

The mobile phase composed of acetonitrile: ammonium acetate buffer, 10 mM (pH 4.0) in a ratio of 50:50 by volume was degassed for 20 min in an ultrasonic bath (Branson Cleaning Equipment Co., USA) prior to use. HPLC system (Shimadzu, Japan) was equipped with a C<sub>18</sub> reversed-phase column (Cosmosil Nacalai Tesque, 5 µm particle diameter, 4.6 × 150 mm, Bondapak, USA).

Chromatography was performed at a flow rate of 1 mL/min at 30°C (16). All solutions were filtered through a 0.45 µm membrane (Sartorius, Germany) prior to use. The channel on the diode-array detector was configured to acquire data at 244 nm. The injection volume was 30 µL. The column was equilibrated for a minimum of 20 min with the mobile phase flowing through the system before injection of the drug standards. The run time was set at 10 min with

the system operating at an air-conditioned temperature of 20°C.

#### 2.4.3. Preparation of stock solutions and standard working solutions

Stock solutions of repaglinide (100 µg/mL) and indomethacin (100 µg/mL) were prepared by dissolving 1 mg of each drug in 10 mL ethanol. The standard solutions were stored at 4°C in a clear glass volumetric flask and light protected with aluminum foil. Repaglinide concentrations in the working solutions used for the calibration curve were 20, 30, 50, 75, 100, 150, and 200 ng/mL. Quality control (QC) samples (of low, medium, and high concentrations) at 40, 80, and 150 ng/mL were prepared in the same way as the calibration standards. These working solutions were prepared fresh daily by making dilutions of the stock solution in ethanol. Working standard solutions of indomethacin were prepared by diluting the stock solution with ethanol at a concentration of 500 ng/mL. Fifty microlitres of indomethacin solution was used for every analysis (8).

#### 2.4.4. Calibration curve

Blank plasma samples were spiked with working standard solutions to obtain final concentrations in the range of 20-200 ng/mL.

#### 2.4.5. Extraction procedure (8)

The internal standard, indomethacin, and repaglinide solutions were added to blank plasma samples in round bottom glass tubes. One milliliter extraction buffer 0.1 M potassium dihydrogen orthophosphate (KH<sub>2</sub>PO<sub>4</sub>, pH 5.9) was added to the tubes. After the mixture was vortexed, 5 mL of ethyl acetate and 50 µL of isoamyl alcohol were added and the pH was adjusted to 7.4 with 2 M NaOH. The tubes were shaken on a rotator for 10 min followed by centrifugation at 3,000 rpm for 30 min. After centrifugation, the ethyl acetate phase was transferred into V-tubes and evaporated to dryness under a stream of nitrogen at 45°C. The dried extract was reconstituted with 70 µL of mobile phase, vortex-mixed and transferred to a clean autosampler vial. Thirty µL of this solution was injected into the HPLC system.

#### 2.4.6. Method validation

Plasma calibration curves were prepared and assayed in triplicate on three different days to evaluate linearity, precision, accuracy, recovery, limit of quantification (LOQ), limit of detection (LOD), and stability.

#### 2.4.7. Pharmacokinetic analysis

The maximum plasma concentration  $C_{max}$  was obtained

directly from the experimental data;  $T_{\max}$  was defined as the first occurrence of  $C_{\max}$ . The terminal elimination rate constant ( $K_{el}$ ) was estimated using least squares regression analysis of plasma concentration time data obtained during the terminal log-linear elimination phase. Individual half-lives ( $t_{1/2}$ ) were calculated as  $0.693/K_{el}$  and mean half-lives were defined as  $0.693/\text{mean } K_{el}$ . The area under the plasma concentration-time curve (AUC) from 0 – time  $t$  ( $AUC_{0-t}$ ) was estimated by linear trapezoidal approximation (17,18). The AUC from  $t$  to infinity ( $AUC_{t-\infty}$ ) were estimated as  $ct, est K_{el}$ , where  $ct, est$  represents the estimated concentration at time  $t$  based on the regression analysis. The total area under the curve ( $AUC_{0-\infty}$ ) was estimated as the sum of ( $AUC_t$ ) plus ( $AUC_{t-\infty}$ ).

#### 2.4.8. Statistical analysis

The pharmacokinetic parameters for both treatments A and B were analyzed using one way analysis of variance using the SPSS computer program.

### 2.5. Biological study design

The study was carried out to determine the hypoglycemic effect of repaglinide (2 mg) from tablets containing the liquisolid compact formula in comparison to the commercial tablet as well as its effect on the blood glucose tolerance test (OGTT) and insulin levels. The study also evaluate the safety and efficacy of the new formula by studying the effect of several potencies of the liquisolid formula (0.5, 1.0, and 2.0 mg) on blood glucose and insulin level as well as the hepatic and renal toxicity of this newly prepared formula.

#### 2.5.1. Experimental animals

Thirty white male rabbits (Boskat breed) weighing from 1.5-2 kg was used as model animals for determining the biological activity of repaglinide. The animals were housed in a standard temperature and humidity controlled room with a 12 h light/dark cycle. The animal had free access to water and a standard diet.

#### 2.5.2. Experimental plan

Rabbits were divided into five groups (six animals each): GP1-Control group, animals received a single oral dose of glucose D (+) (2 g/kg BW) (glucose load) using the special oral syringe; GP2-Commercial drug group, animals received the glucose load (2 g/kg BW) + the oral dose of commercial drug (2 mg); GP3-Liquisolid compact group (0.5 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisolid compact formula (0.5 mg); GP4-Liquisolid compact group (1.0 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisolid

compact formula (1.0 mg); GP5-Liquisolid compact group (2.0 mg), animals received the glucose load (2 g/kg BW) + the oral dose of the liquisolid compact formula (2.0 mg).

#### 2.5.3. Blood sampling

Blood samples (2 mL) were withdrawn from the animals after 0, 15, 30, 45, 60, 90, and 120 min from retro-orbital plexuses according to the method of Shermer (19) and left to coagulate.

#### 2.5.4. Blood glucose and glucose tolerance test (OGTT)

After overnight food privation, fasting blood glucose was determined in all groups. Simple, direct, colorimetric and automation-ready procedures for measuring glucose were performed using the BioAssay Systems' glucose assay kit, (QuantiChrom™ Glucose Assay Kit), according to Yoon and Mekalanos (20).

Glucose tolerance tests were carried out after glucose load in group 1, after glucose load with commercial drug in group 2, and after glucose load and the new formula drug in other groups 3, 4, and 5. The blood samples were withdrawn for determination of blood sugar after 15, 30, 45, 60, 90, and 120 min. The blood sugar levels were then plotted against time as blood glucose tolerance curves. The first point (zero) of these curves represents the fasting blood sugar level. Insulin levels were also determined at each time interval.

The area under the curve (AUC) of change in blood glucose was determined using the following equation:

$$AUC = \Sigma \{[(C_n - C_0) + (C_{n+1} - C_0)] \times (t_{n+1} - t_0)\}$$

#### 2.5.5. Biochemical parameters

Insulin levels were also determined at each time interval using Calbiotech Insulin ELISA kit based on direct sandwich technique according to the method of Beischer (21). Urea was determined using a colorimetric urea kit (Dam method) according to the method of Wybenga (22). Creatinine was colorimetrically quantified using the QuantiChrom™ Creatinine Assay Kit according to Wang *et al.* (23). SGPT was determined using a UV kinetic method kit according to the method of Herny (24).

### 2.6. Statistical analysis

Two types of statistical tests were performed in this study, Student "t" test and the analysis of variance "Two-way ANOVA" according to the methods of Campbell (25) and Bailey (26). The data are expressed as mean  $\pm$  SD and the values of  $p < 0.05$  were considered statistically significant.



### 3. Results and Discussion

#### 3.1. Method validation for bioavailability

##### 3.1.1. Calibration curves, precision, accuracy, and linearity

The calibration curve for repaglinide was linear in the concentration range of 20-200 ng/mL in rabbit plasma. The correlation coefficient of the line was 0.9930. The precision and accuracy of the assay were determined from the low (40 ng/mL), medium (80 ng/mL), and high (150 ng/mL) QC plasma samples. Inter-day assays were determined by analyzing QC samples in triplicate and were analyzed on three different days. Intra-day assays were determined for each QC sample in plasma, each in triplicate on one day. Intra and inter-day precision in this study was expressed as percent of coefficient of variation (CV). Accuracy was expressed as the mean percentage of analyte recovered in the assay (27). According to center for Drug Evaluation Research (CDER) (28), the precision determined at each concentration should not exceed 15% of CV except for LOQ, where it should not exceed 20% of the CV. The results of the precision and accuracy determined by the intra- and inter-day method are shown in Table 2.

##### 3.1.2. Recovery

The recovery assay was assessed by comparing the

peak area ratio (repaglinide/indomethacin) obtained from spiked plasma samples of different repaglinide concentrations (20-200 ng/mL) to the peak area ratios for the samples containing the equivalent amounts of the drug and internal standard directly dissolved in the mobile phase. Three replicate samples were measured at each drug concentration and the % average recovery study for repaglinide in plasma was 107.6%.

##### 3.1.3. Stability

Repaglinide was found to be stable in rabbit plasma (< 5% loss) after three freeze/thaw cycles.

#### 3.2. Pharmacokinetic study

Mean plasma concentration-time profiles in treatments A and B are presented in Table 3 and Figure 1. The mean peak time ( $T_{max}$ ) in treatments A and B is 1 hour and the average peak plasma concentration values ( $C_{max}$ ) were 125 and 70 ng/mL, respectively. The mean area under the plasma concentration-time curve ( $AUC_{0-6}$ ) values were 373.5 and 190.38 ng-hr/mL and  $AUC_{0-\infty}$  values were 470.78 and 226.69 ng-hr/mL, respectively.

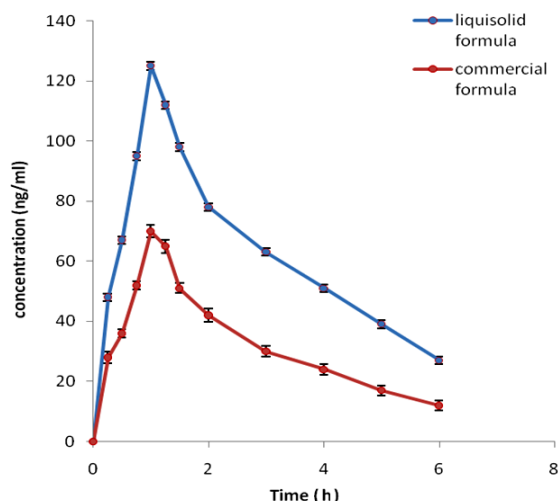
Statistical analysis of the pharmacokinetic data revealed that there were significant differences between treatments A and B in  $C_{max}$ ,  $K_{el}$ ,  $t_{el}$ ,  $TCR$ ,  $AUC_{0-6}$ ,  $AUC_{0-\infty}$ ,  $AUMC_{0-6}$ ,  $AUMC_{0-\infty}$ , and  $MRT$ . Also, there was an insignificant difference in  $T_{max}$ , while there was a significant difference in  $C_{max}/AUC_{0-6}$ .

**Table 2. Intra-and inter-day accuracy and precision of the assay method**

	Nominal concentrations (ng/mL)	Mean concentrations observed (ng/mL)	Accuracy (%)	Precision (%)
Inter-day	40	37	92.5	6.7
	80	73.53	92.29	4.73
	150	151	100.66	5
Intra-day	40	36.9	92	8.3
	80	72.15	90.187	4.85
	150	135	90	12

**Table 3. t-Test independent for comparison between liquisolid formula and commercial product of ripaglinide**

Items	Liquisolid formula			Commercial product			p
	Mean	SD	SE	Mean	SD	SE	
$C_{max}$ (ng/mL)	125	2.915	1.304	70	4.743	2.121	< 0.0001
$T_{max}$ (h)	1	0.07211	0.03225	1	0.04743	0.02121	1
$K_{ab}$ ( $h^{-1}$ )	1.76663	0.00152	0.0006796	1.768	0.005856	0.002619	0.103
$t_{(1/2)ab}$ (h)	0.3931	0.0003384	0.0001513	0.3919	0.001294	0.0005789	0.1024
$K_{el}$ ( $h^{-1}$ )	0.2776	0.002842	0.001271	0.3306	0.007362	0.003293	< 0.0001
$t_{(1/2)el}$ (h)	2.497	0.02557	0.01144	2.097	0.04678	0.02092	< 0.0001
$TCR$ (mL/min)	0.07388	0.393	0.753	0.1558	0.001187	0.000531	< 0.0001
$AUC_{0-6}$ (ng•hr/mL)	373.5	2.069	0.9253	190.38	3.321	1.485	< 0.0001
$AUC_{0-\infty}$ (ng•h/mL)	470.78	1.086	0.4855	226.69	2.513	1.124	< 0.0001
$AUMC_{0-6}$ (ng•h <sup>2</sup> /mL)	936.56	2.126	0.9507	447.1	12.539	5.608	< 0.0001
$AUMC_{0-\infty}$ (ng•h <sup>2</sup> /mL)	1520.2	3.869	1.73	670.36	1.792	0.6673	< 0.0001
$MRT$ (h)	3.229	0.01551	0.006938	2.958	0.03929	0.01757	< 0.0001
$C_{max}/AUC_{0-6}$ ( $h^{-1}$ )	0.3346	0.005965	0.002668	0.3674	0.01851	0.008279	0.0055



**Figure 1.** Mean plasma level of repaglinide in rabbits following oral administration of liquisolid formula and commercial product. Each value represents the mean  $\pm$  SD ( $n = 6$ ).

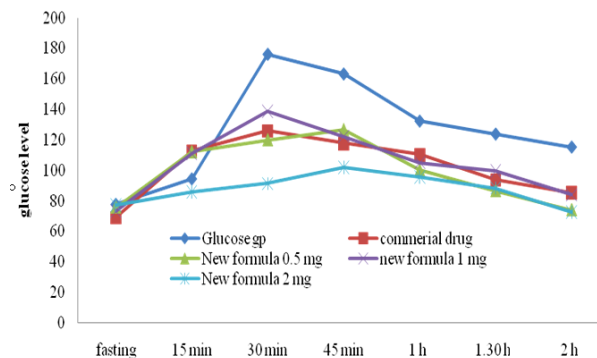
### 3.3. Biological study

#### 3.3.1. Blood glucose tolerance

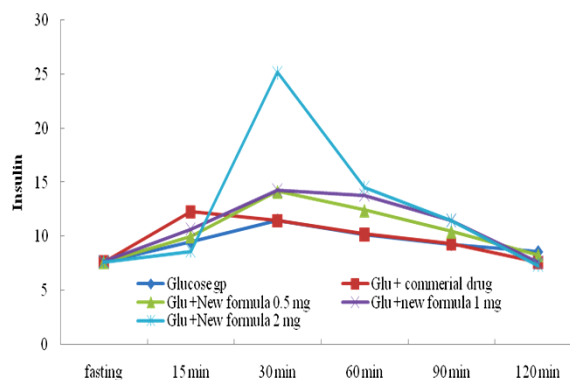
As shown in Table 4 and Figure 2, the blood glucose time course change data revealed that the new liquisolid compact formula exerted a significant hypoglycemic effect compared to the repaglinide commercial product. The percent change glucose level, as compared with that in the glucose group, 30.8% and 19.1% in liquisolid compact (2 mg) and commercial product, respectively. Similarly, the other two potencies of the new compact formula exhibited a percent change glucose level of 21.3% and 16.9% at 0.5 mg and 1 mg, respectively.

#### 3.3.2. Insulin

The time change of insulin, as shown in Table 4 and Figure 3, showed a significant increase in insulin levels in groups treated with the new liquisolid compact formula compared with the commercial product. The percent changes were 3.2, 11.2, 15.8, and 32.2% in repaglinide commercial product (2 mg), liquisolid



**Figure 2.** Time course changes of blood glucose level (mg/dL) in commercial drug group as well as new drug formula groups after administration of glucose load.



**Figure 3.** Time course changes of insulin level (ng/mL) in commercial drug group as well as new drug formula groups after administration of glucose load.

compact formula (0.5, 1, and 2 mg), respectively, compared with that in the glucose group.

#### 3.3.3. Area under the curve

Table 5 shows the AUC of blood glucose in the rabbit groups treated with glucose only and glucose + commercial product (2 mg), and glucose + liquisolid compact formula (2 mg), respectively. The statistical study revealed that the main two effects (liquisolid compact formula and time course) were significant ( $p < 0.05$ ) for AUC. For the glucose new formula rabbit group, the AUC was significantly lower than that in the

**Tables 4.** Time course change of blood glucose and insulin levels in all groups after a glucose load (group 1) or glucose load-repaglinide commercial product, 2 mg (group 2) or glucose load-liquisolid compact formula, 0.5, 1, and 2 mg (groups 3, 4, and 5)

	Group 1	Group 2	Group 3	Group 4	Group 5
Glucose levels (mg/dL)					
Mean	126.1	101.9	99.21	104.80	87.29
SD	35.0	20.1	21.12	22.28	10.17
SE	13.2	7.6	7.98	8.42	3.84
% of change	–	19.1%	21.3%	16.9%	30.8%
Insulin levels (ng/mL)					
Mean	9.41	9.72	10.48	10.90	12.45
SD	1.32	1.95	2.47	2.91	6.81
SE	0.54	0.80	1.01	1.19	2.78
% of change	–	3.2%	11.3%	15.8%	32.2%

**Table 5. Blood glucose calculated from area under the curves of blood concentration at specific time periods**

Time (min)	Glucose group			Commercial group			Liquisolid compact formula group		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
0-15	1,294	27	11	1,378	53	21	1,197	39	16
15-30	2,045	29	12	1,779	28	11	1,330	39	16
30-45	2,595	49	20	1,817	38	16	1,462	35	14
45-60	2,243	54	22	1,669	55	22	1,478	21	8
60-90	3,785	63	26	2,977	107	44	2,703	44	18
90-120	3,500	47	19	2,640	72	29	2,357	45	18

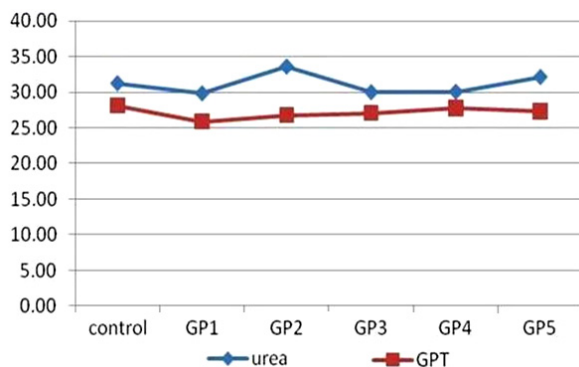
**Table 6. Biochemical parameters in treated groups in comparison with control group**

	Control	Group 1	Group 2	Group 3	Group 4	Group 5
Urea (mg/dL)	31.31	29.92 ± 2.8	33.62 ± 4.3	30.09 ± 3.1	30.14 ± 5.4	32.19 ± 2.7
SGPT (U/L)	28.11 ± 3.5	25.91 ± 2.1	26.79 ± 2.3	27.09 ± 2.9	27.79 ± 2.3	27.32 ± 3.1
Creatinine (mg/dL)	0.15 ± 0.019	0.13 ± 0.008	0.21 ± 0.1	0.14 ± 0.008	0.14 ± 0.01	0.14 ± 0.01

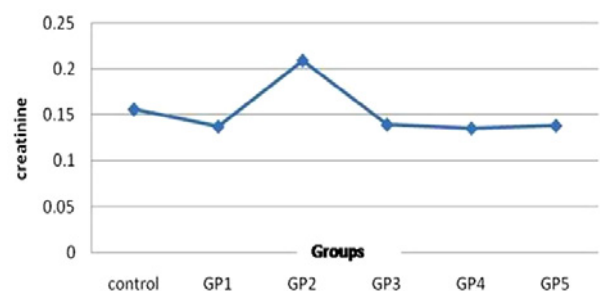
**Table 7. ANOVA analysis of the tested parameters**

		SS	DF	MSS	F calculated
Glucose	Between groups	5,390	2	2,695	4.67
	Within groups	10,390	18	577	
	Total	15,780	20		
Insulin	Between groups	2,017	2	1,009	58.3
	Within groups	259.3	18	17.3	
	Total	2,277	20		
Urea	Between groups	65.2	5	32.6064625	2.17
	Within groups	451.5	30	15.049145	
	Total	516.7	35		
Creatinine	Between groups	0.0249	5	0.012452611	2.46
	Within groups	0.1516	30	0.005054133	
	Total	0.1765	35		
SGPT	Between groups	18.1	5	9.062	1.18
	Within groups	230.9	30	7.697	
	Total	249.0	35		

Abbreviations: SS, sums of squares; DF, degrees of freedom; MSS, mean sums of squares.



**Figure 4. Biochemical parametrs changes in treated groups as copmared with control group.**



**Figure 5. Ceratinine level (mg/dL) changes in treated groups as compared with control group.**

G group ( $p < 0.05$ ) in the periods 15-30, 30-45 min, and 45-90 min. The total AUC was also significantly lower in the glucose + liquisolid compact formula (2 mg) group than that in the G group ( $p < 0.05$ ).

3.3.4. Biochemical parameters

As shown in Tables 6 and 7, and Figures 4 and 5, the new liquisolid compact formula did not exert any significant changes in the levels of urea, creatinine, and SGPT in all studied groups.

#### 4. Conclusion

On the basis of the previous findings, it was concluded that the modified HPLC method used was rapid, simple, and sensitive for quantification of repaglinide in plasma samples. The method has good linearity, accuracy, precision, selectivity, and stability over the relevant therapeutic range. Also, the bioavailability of repaglinide was improved significantly when administered orally as the liquisolid compact preparation. In turn, the new liquisolid formula controls blood sugar more efficiently than the commercial product by stimulating the release of insulin from the  $\beta$ -cell of the pancreas.

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**Original Article****Evaluation of estrogen receptor *alpha*, estrogen receptor *beta*, progesterone receptor, and cKIT expression in desmoids tumors and their role in determining treatment options****Gabriel A. C. Santos<sup>1</sup>, Isabela W. Cunha<sup>2</sup>, Rafael M. Rocha<sup>2,\*</sup>, Celso A. L. Mello<sup>3</sup>, Gustavo C. Guimarães<sup>1</sup>, José H. Fregnani<sup>1</sup>, Ademar Lopes<sup>1</sup>**<sup>1</sup> Department of Pelvic Surgery, Cancer Hospital A.C. Camargo, São Paulo, Brasil;<sup>2</sup> Department of Anatomic Pathology, Cancer Hospital A.C. Camargo, São Paulo, Brasil;<sup>3</sup> Department of Clinical Oncology, Cancer Hospital A.C. Camargo, São Paulo, Brasil.**Summary**

The present study evaluates the protein expression of estrogen receptor *alpha* (ER $\alpha$ ), estrogen receptor *beta* (ER $\beta$ ), progesterone receptor (PR) and cKIT in a wide number of desmoids tumors and their role in determining treatment options. Fifty-nine cases classified as muscle aponeurotic fibromatosis were selected. Samples were grouped by tumor location in: head and neck, extremity and abdominal/trunk; type of resection of the primary tumor (complete resection with adequate margins, marginal resection and resection with inadequate margins); type of treatment (exclusive surgery, surgery followed by radiation therapy and surgery followed by tamoxifen or cyclooxygenase inhibitor). A tissue microarray (TMA) was built and the immunohistochemical reactions were performed against ER $\alpha$ , ER $\beta$ , PR, and c-kit. All cases were negative for ER $\alpha$ , PR and c-KIT. 53/59 cases were positive for ER $\beta$ . No significant difference was observed among clinical variables and the ER $\beta$  status. The estimated 5 and 10 year local recurrence free survival (LRFS) for the patients with complete or marginal resection was 75% and 75%, respectively. Tumor location ( $p = 0.006$ ) and type of resection ( $p = 0.001$ ) were predictive of local relapse in the univariate analysis. All patients treated with post-operative tamoxifen were LRFS ( $p = 0.035$ ). Head and neck and extremities lesions showed higher recurrence rates compared to abdominal/trunk lesions. Marginal resection was associated with local recurrence. In conclusion, although this is a retrospective study, the results presented can contribute to better understanding of the mechanisms under desmoid tumor development and can propose tamoxifen as a therapeutic option to be tested in prospective trials.

**Keywords:** Immunohistochemistry, hormone receptors, desmoids tumors**1. Introduction**

Desmoid tumors, also known as muscle-aponeurotic fibromatosis, present locally aggressive fibroblastic proliferation. It arises in deep soft tissues and is characterized by local invasion and high rates of local recurrence. However, no metastatical potential

is observed. The incidence of desmoid tumors is 2 to 4 cases/1,000,000, and it is more frequent in female gender (1). Complete surgical resection of primary tumor is associated with best outcomes and death is a late event in the course of the disease and is the result of local destruction and complications.

Most of patients with desmoids tumors have long term survival even with recurrent disease. Unresectable disease is usually treated with radiation therapy, chemotherapy or hormone therapy. The results of such treatments are disappointing due to low response rates. Hormone therapy has been explored as a strategy to disease control showing null to satisfactory

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response rates (2-5). The inhibition of cKIT by imatinib represents another therapeutic option which can be employed in the control of muscle aponeurotic fibromatosis (4). Data related to estrogen receptor expression as well as expression of other molecules such as progesterone receptor (PR) and cKIT and its relation to clinical outcome and treatment response is controversial (6,7).

The aim of the present study was to analyze the protein expression of estrogen receptor *alpha* (ER $\alpha$ ), estrogen receptor *beta* (ER $\beta$ ), PR and cKIT in a wide number of desmoids tumors treated in a same institution and their role in determining treatment options.

## 2. Methods

### 2.1. Patients and samples

Fifty-nine cases classified as muscle aponeurotic fibromatosis based on the WHO classification were selected (8). Samples were grouped by tumor location in: head and neck, extremity and abdominal/trunk; type of resection of the primary tumor (complete resection with adequate margins, marginal resection and resection with inadequate margins); type of treatment in: exclusive surgery, surgery followed by radiation therapy and surgery followed by tamoxifen or cyclooxygenase (COX) inhibitor.

### 2.2. TMA construction and immunohistochemistry

A tissue microarray containing 59 cases spotted in duplicate was built for the immunohistochemical study (9). The immunohistochemical reactions were performed using a polymeric biotin-free detection system (Advance, DAKO™) according to the company guideline and standard protocols using the following antibodies:  $\alpha$ -estrogen (clone SP1, NeoMarkers®) working dilution 1/5,000,  $\beta$ -estrogen receptor (polyclonal, Chemicon®) working dilution 1/400, PR (clone 636, DAKO®) working dilution 1/2,000, and c-kit (polyclonal, DAKO®) working dilution 1/200. Cases were considered positive when at least 1% of tumor cells showed moderate nuclear staining (Allred scoring system).

### 2.3. Statistical analysis

Statistical analysis was performed using SPSS program for Windows (version 8, SSPS Inc., Chicago, IL, USA). The Kaplan-Meier method was used for actuarial survival estimates. The multivariate analysis was performed using the proportional COX model of regression by the method of stepwise-forward. Local Recurrence Free Survival (LRFS) was calculated from the time of complete or marginal resection until detection of recurrence. Patients with inadequate surgery were excluded from the analysis.

## 3. Results

The median age for the 78 patients was 30. Forty-eight patients were female and 30 male. Sixty-six patients had no previous treatment and 12 had previous biopsy or incomplete surgery. According to anatomical distribution, 31 patients had lesion located in abdominal/trunk region, 37 in extremities and 10 in head and neck region. Considering surgical procedures 40 patients had adequate surgery with disease free margins, 21 patients had marginal resection of the tumors and 17 patients had inadequate surgery with tumors achieving margins. After admission at our institution, 57 patients were treated with exclusive surgery and 21 received combined treatment consisting of surgery followed by tamoxifen (15 patients), and surgery followed by radiotherapy (6 patients) (Table 1). The recurrence rate for the 61 patients with complete and marginal resection was 24.6% (Table 1).

Immunohistochemical study was conducted in 59 cases adequately represented in TMA spots. All cases were negative for ER $\alpha$ , PR and c-KIT expression. Fifty three out of 59 cases were positive for ER $\beta$ . No significant difference was observed among clinical variables and the ER $\beta$  status as shown in Table 2.

The estimated 5 and 10 year LRFS for the patients with complete or marginal resection was 75% and 75%, respectively (Figure 1). Tumor location ( $p = 0.006$ ) and type of resection ( $p = 0.001$ ) were predictive of local relapse in the univariate analysis. LRFS rates for each clinical variable are shown in Table 3. The actuarial

**Table 1. Distribution of clinical variables for the 78 patients with fibromatosis**

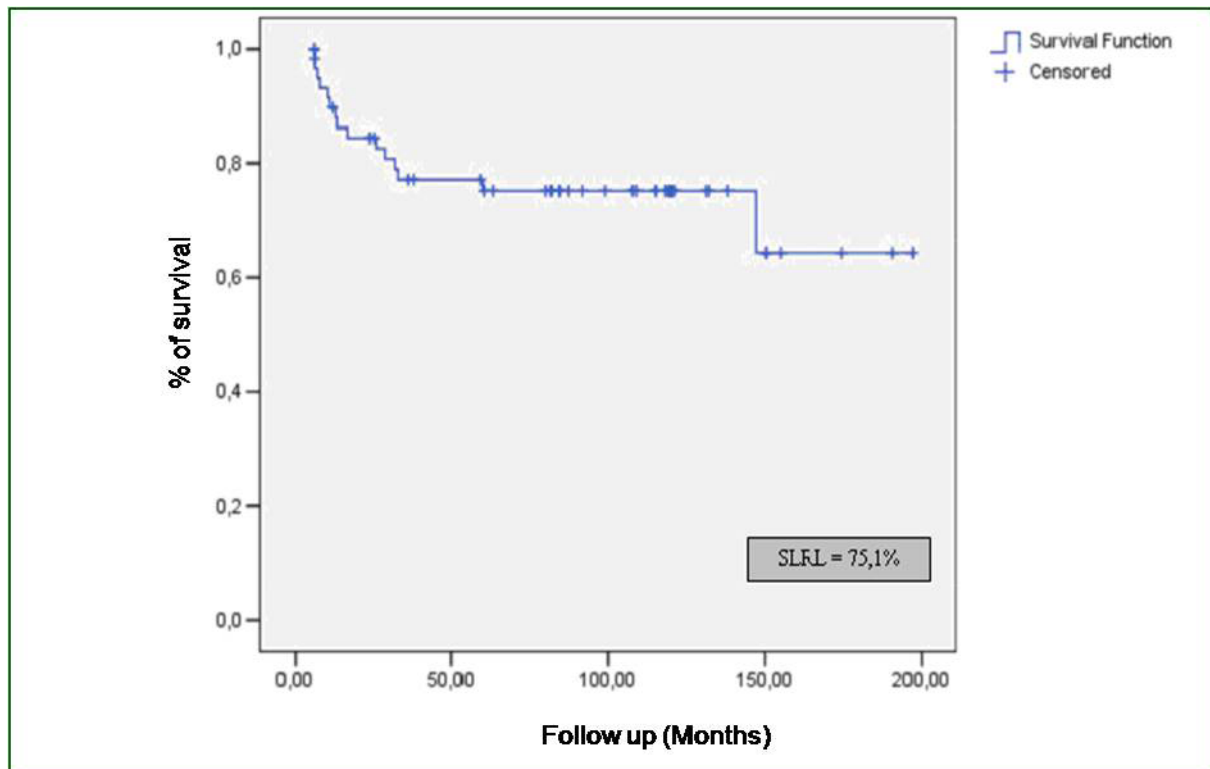
Variable	Category	Number of cases
Age (years)	< 50	38
	> 50	40
Median (years)	30	
Sex	Male	30
	Female	48
Situation at admission	Not treated	66
	Biopsy or incomplete Surgery	12
Tumor site	Abdomen /visceral	31
	Extremities	37
	Head and neck	10
Type of resection	Adequate	40
	Marginal	21
	Inadequate	17
Type of treatment	Exclusive surgery	57
	Combined Treatment	
	Surgery + radiotherapy	6
	Surgery + tamoxifen	15
Local recurrence*	No	46
	Yes	15

\*Patients with complete and marginal resection ( $n = 61$ ).

**Table 2. ERβ status versus clinical variables for 59 patients with fibromatosis**

Variable	Category	ERβ		p
		Negative n (%)	Positive n (%)	
Age (years)	< 50	3 (10)	27 (90)	1.00
	> 50	3 (10.3)	26 (89.7)	
Sex	Male	3 (13)	20 (87)	0.669
	Female	3 (8.3)	33 (91.7)	
Admission situation	Not treated	5 (10)	45 (90)	1.00
	Biopsy or incomplete surgery	1 (11.1)	8 (88.9)	
Tumor site	Abdomen/cavity	3 (13)	20 (87)	-*
	Extremities	2 (7.4)	25 (92.6)	
	Head and neck	1 (11.1)	8 (88.9)	
Type of resection	Adequate	3 (9.4)	29 (90.6)	-*
	Marginal	2 (12.5)	14 (87.5)	
	Inadequate	1 (10)	10 (90)	
Type of treatment	Exclusive surgery	3 (7.1)	39 (92.9)	-*
	Surgery + tamoxifen	3 (27.3)	8 (72.7)	
	Surgery + tamoxifen + COX inhibitor	0 (0)	1 (100)	
	Surgery + radiotherapy	0 (0)	5 (100)	
Local recurrence	No	4 (9.3)	39 (90.7)	0.658
	Yes	2 (12.5)	14 (87.5)	
Post-operative tamoxifen	No	3 (6.4)	44 (93.6)	0.092
	Yes	3 (25)	9 (75)	
Post-operative radiotherapy	No	6 (11.1)	48 (88.9)	1.00
	Yes	0 (0)	5 (100)	

\* Pearson or Chi-square test could not be applied.



**Figure 1. Local recurrence free survival in months for 61 patients with fibromatosis.**

LRFS curves for variables with statistical significance are shown in Figures 2 and 3. LRFS was 100% for the group of patients treated with post-operative tamoxifen ( $p = 0.035$ ) (Figure 4).

In the multivariate analysis, head and neck and extremities lesions had higher recurrences rates compared to abdominal/trunk lesions. Marginal resection was associated with local recurrence (Table 4).

#### 4. Discussion

Muscle aponeurotic fibromatosis or desmoids tumors are rare neoplasms that arise in soft tissues. The

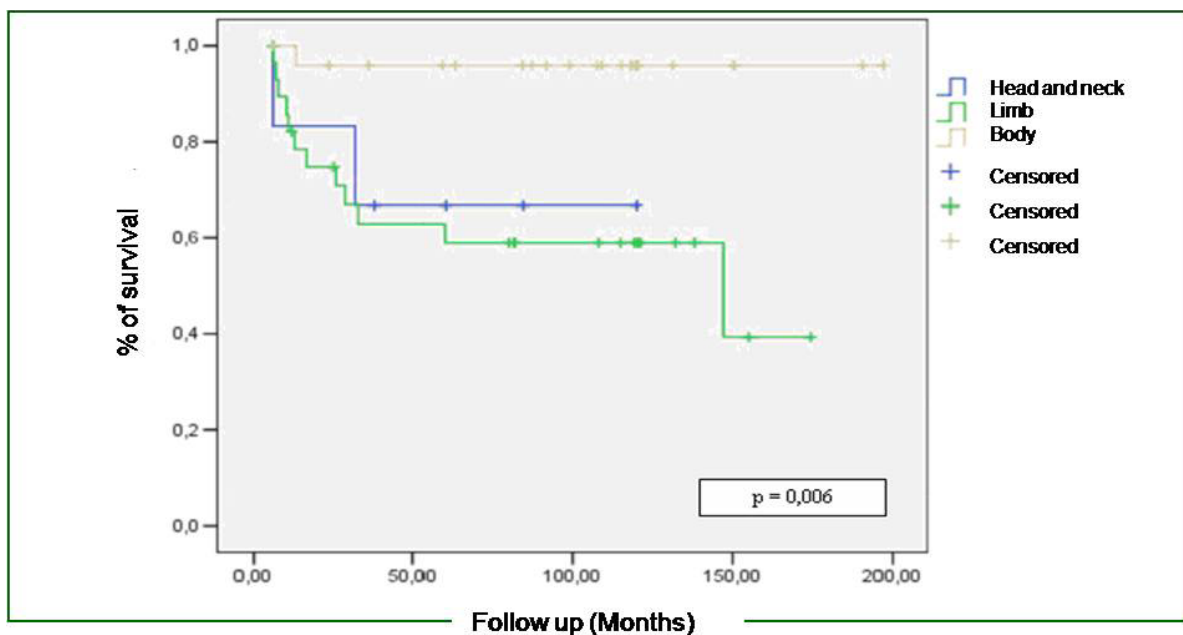
pathogenesis of these tumors is not well understood and the hormone sensitivity of desmoids still controversial. The expression of hormone receptors and cKIT has been extensively studied in desmoids-type fibromatosis due to the fact that these molecules could be used as therapeutic targets.

Immunohistochemical studies on desmoids tumors have shown contradictory findings on hormone receptor status. Our study detected high rate of ER *beta* receptor expression (89%) but no ER *alpha* (0%). More interestingly we found that patients treated with tamoxifen had a much better outcome than those treated by surgery alone or radiation, with LRFS of 100%

**Table 3. Local recurrence free survival at 5 and 10 years according to clinical variables for the 78 patients with fibromatosis**

Variable	Category	<i>n</i>	LRFS in 5 years (%) <sup>*</sup>	LRFS in 10 years (%) <sup>*</sup>	<i>p</i> <sup>**</sup>
Age (years)	< 50	38	73.8	73.8	0.726
	> 50	40	77.4	77.4	
Sex	Male	30	66.7	66.7	0.123
	Female	48	81.6	81.6	
Admission situation	Not treated	66	72.9	72.9	0.236
	Biopsy or incomplete surgery	12	62.5	62.5	
Location	Abdomen/cavity	31	95.8	95.8	0.006
	Extremities	37	59.0	59.0	
	Head and neck	10	66.7	66.7	
Type of resection	Adequate	40	88.9	88.9	0.001
	Marginal	21	49.6	49.6	
	Inadequate	17	— <sup>***</sup>	— <sup>***</sup>	
Type of treatment	Exclusive surgery	57	71.3	71.3	0.345
	Combined treatment	21	83.3	83.3	

<sup>\*</sup> Only patients with adequate and marginal resection ( $n = 61$ ); <sup>\*\*</sup> *p* value by Log-rank test; <sup>\*\*\*</sup> Not applicable to patients with inadequate resection.



**Figure 2. Local recurrence free survival in months for 61 patients with fibromatosis considering type of resection.**

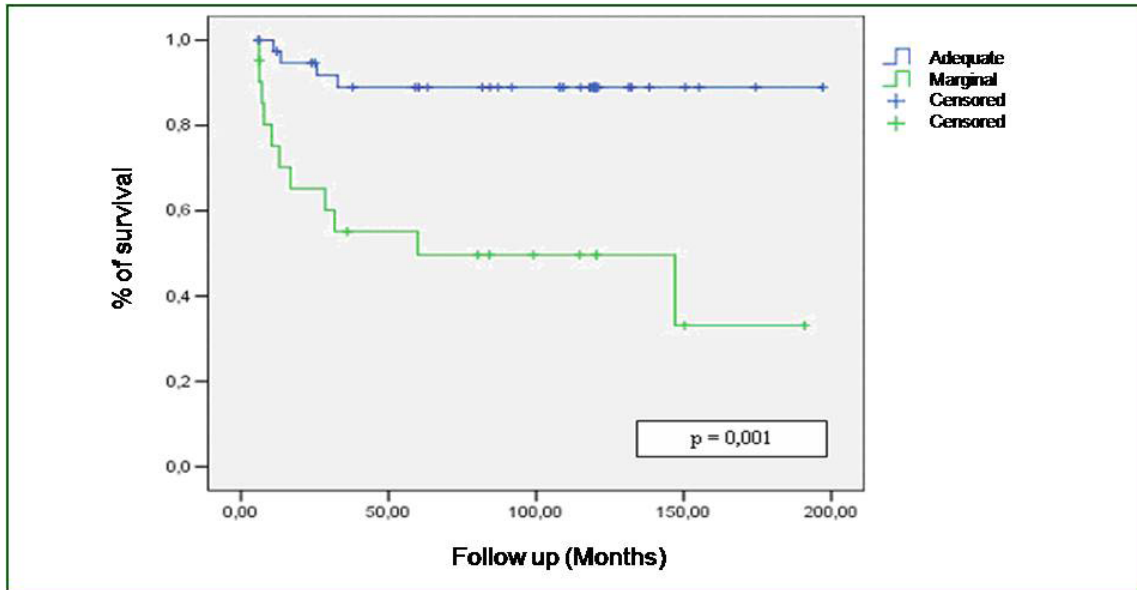


Figure 3. Local recurrence free survival in months for 61 patients with fibromatosis considering topography.

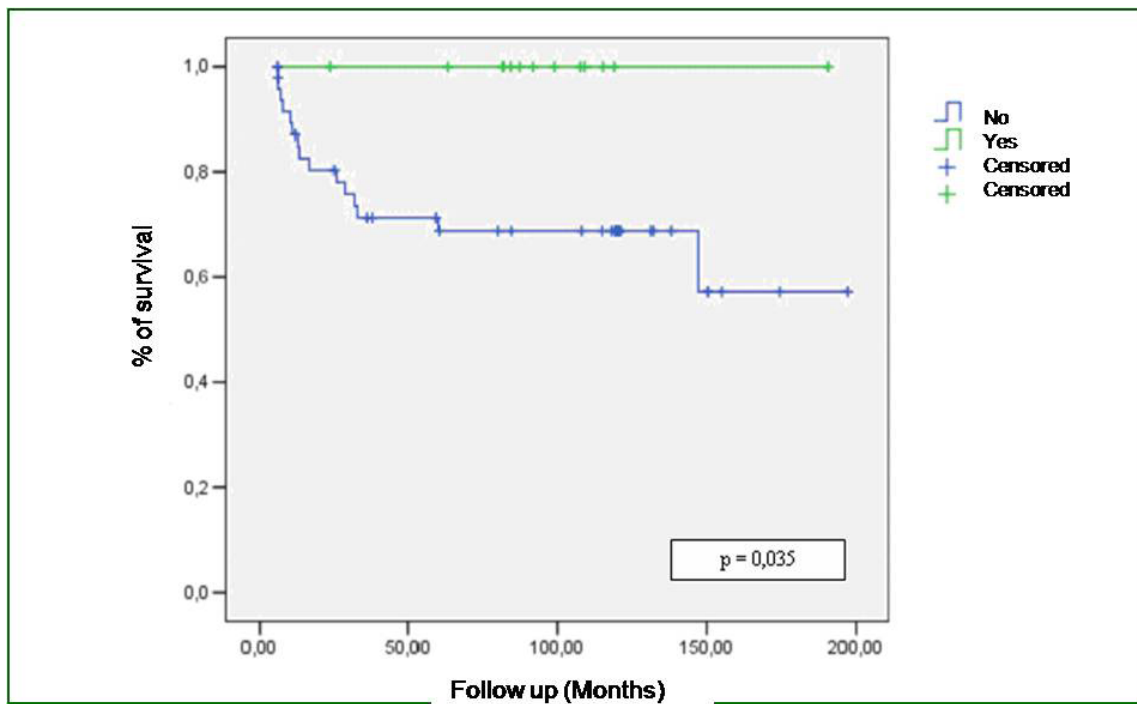


Figure 4. Local recurrence free survival in months for 61 patients with fibromatosis considering pos-operative tamoxifen.

Table 4. Risk factors for local recurrence in patients with fibromatosis

Variable	Category	p*	Hazard ratio (HR)	95% CI
Location	Abdomen/cavity (reference)	–	1.00	–
	Superior extremity	0.043	10.49	1.07 – 102.84
	Inferior extremity	0.036	9.22	1.15 – 73.72
	Head and neck	0.049	11.32	1.00 – 127.19
Type of resection	Adequate (reference)	–	1.00	–
	Marginal	0.003	6.33	1.88 – 21.22

\*COX regression.

at 5 and 10 years ( $p = 0.035$ ), although only a small group of patients received tamoxifen and most of these patients had abdominal/trunk tumors. Nine out of the 12 patients treated with tamoxifen were ER $\beta$  positive (75%) and 3 were ER $\beta$  negative (25%) ( $p = 0.092$ ). ER $\beta$  expression was not associated with better outcome neither with response to tamoxifen in our study, but we should emphasize that the characteristic and the size of the sample of patients were underpowered to show an improvement in outcomes for this low grade disease.

Tamoxifen is the most commonly agent used in hormone manipulation for breast cancer and other estrogen sensitive tumors (3,7). Tamoxifen is a selective estrogen receptor (ER) modulator that acts *via* binding on ER *alpha* and inhibits cell proliferation (3). Most of the studies have shown that desmoids tumor are ER $\alpha$  negative. Few studies have analyzed ER $\beta$ . Leithner A *et al.* (6) reported immunohistochemical analysis on 80 desmoid tumors and all cases were negative for ER $\alpha$  and PR and less than 10% (7/80) of cases were positive for ER $\beta$ . Deyrup *et al.* (7) showed 100% positivity of ER $\beta$  in 40 cases of extra-abdominal fibromatosis and no expression of ER $\alpha$ . Our results could contribute to explain or better understand the hormone sensitivity of these tumors in the scenario of negative ER $\alpha$ . The mechanisms responsible for tumor response to therapy with imatinib must be identified (4). In accordance with previous data, the present did not detect immunohistochemical expression of cKIT.

In summary, we detected high percentage of ER $\beta$  expression in desmoids and, in agreement with the literature, no expression of ER $\alpha$  and cKIT was observed. The observation, in this study, that adjuvant tamoxifen therapy is protective after adequate or marginal resection of desmoids tumor is also relevant. Although this is a retrospective study, the results presented can contribute to better understanding of the mechanisms under desmoid tumor development and can

propose tamoxifen as a therapeutic option to be tested in prospective trials.

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**Original Article**

# The healing effect of electrolytic-reduction ion water on burn wounds

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**Summary**

We prepared a lotion using electrolytic-reduction ion water (ERI), and evaluated the healing effects of this lotion (ERI lotion) on burn wounds. Third degree burn wounds were induced in the mouse dorsal skin, and ERI lotion or physiological salt (PS) lotion was applied to the wounds from immediately after injury [ERI (+) group and ERI (-) group as a control group, respectively]. The burn wound area was measured, and its serial changes were evaluated. In addition, histological examination of the burn wound site (on day 3) was performed. Comparison of the ERI (+) and (-) groups showed a significant reduction in the burn wound area in the former. Histological examination confirmed many interstitial spaces, blood vessels, and lymphatic vessels in the subcutaneous tissue in the ERI (-) compared with the ERI (+) group. These results suggest the promotion of burn wound healing by ERI lotion.

**Keywords:** Electrolytic-reduction ion water, burn wound healing, burn wound, edema, subcutaneous tissue

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## 1. Introduction

Wound healing is a phenomenon in which regeneration or wound repair reactions occur in tissues and cells that have become defective due to tissue injury such as by trauma. The types of trauma include incised, contused, or stab wounds, burns, and chemical injuries. The series of biological reactions by which the human body attempts to repair and reconstruct tissues themselves and functions that have been lost by the above mechanisms is called wound healing (1). Among the types of trauma, burns are skin injuries caused by heat, classified according to their depth of the burn degree into: First-degree, injury involving only the skin surface; second-degree, injury that also involves the dermis and is often accompanied by edema; and third-degree, injury involving the dermis and subcutaneous tissue and often resulting in scar formation after healing; fourth-degree, injury involving the deep structures, such as muscle,

tendon, bone, *etc.* (2). For second and third degree burns, the main treatment method was conventionally local therapy with drugs (such as disinfectants and ointments containing steroids or antibiotics), but has been gradually changed to sealing therapy with various wound dressings (such as polyurethane film, hydrocolloid, polyether form, and hydro-gel, alginate dressing, or hydro-polymer) to promote biological repair mechanisms (3,4). These drugs for local therapy and wound dressings play important roles such as the promotion of epithelialization, prevention of infection, and reduction of pain in injured areas. Recently, basic fibroblast growth factor (bFGF) preparations have also been clinically applied, shortening the burn wound healing period (5-8).

We have performed some studies on the characteristics of ERI (9-11). ERI is water containing a large amount of electrons through the electrolysis of natural water, followed by electric current/pressure application using a special diaphragm system. ERI shows cleaning, deodorant, antimicrobial, and antidust effects because dirt and bacteria, as the causes of odor, are detached and removed by its specific alkaline property and negatively charged ions (9). This water also has rust-preventing and anti-septic effects. In addition, stable emulsions could be prepared by

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emulsification of various types of oil using ERI alone without using emulsifiers, showing its emulsifying effect (10). Taking advantages of these properties, ERI is widely used at present as a cleaning agent incorporated in various industrial products.

We previously prepared magnesium aluminum silicate (Smectone<sup>®</sup>) gels using ERI as a dispersal medium for medical drugs, and evaluated their physiochemical properties (12,13). As a result, the use of ERI compared with purified water facilitated the preparation of drug delivery system (DDS) drugs with the maintenance of the gel state. These results showed that the use of gels with functions being maintained using the specific properties of ERI is useful for preparing percutaneously absorbed drugs such as sustained-release preparations.

In this study, to facilitate the application of ERI as a component of medical drugs, we evaluated the healing effects of this water on burn wounds.

## 2. Materials and Methods

### 2.1. Animals

The present study was approved by the Institutional Review Board, Meiji Pharmaceutical University, using 14 male ddY mice aged 7 weeks (Sankyo Labo Service Co., Japan).

### 2.2. Materials and reagents

As ERI, S-100<sup>®</sup> (A.I. System Product Co., Japan) was used. All reagents were of special grade.

### 2.3. Preparation of ERI and PS lotions

For the preparation of ERI and PS lotions, sodium carboxymethylcellulose (cmc-Na) (Kanto Chemical Co., Inc., Tokyo, Japan) was added to each dispersal medium (ERI or PS) to adjust the final concentration to 7%.

### 2.4. Induction of burns

Under anesthesia induced by the intraperitoneal injection (0.05 mg/g, *i.p.*) of pentobarbital sodium (Nacalai Tesque, Inc., Kyoto, Japan), the back of mice after 24 h fasting was shaved using electric clippers, and hair was removed using a depilatory cream based on the reports by Papp *et al.* and Kaneko *et al.* (14,15). Third degree burn was induced by applying the tip of an electric soldering iron (HAKKO FX-951, Hakko Co., Osaka, Japan) at 300°C to the mouse back for 5 seconds. The side of the tip of the soldering iron was evenly applied so that the pressure at the time of burn induction was constant. To avoid variations between experiments, the same researcher produced all burns.

### 2.5. Measurement of the burn wound area

Burn wounds were induced in 12 mice, and the mice were randomly divided into two groups (6 mice each) undergoing the application of ERI lotion [ERI (+) group] or PS lotion [ERI (-) group]. Immediately after wound induction (day 0), the wound was photographed using a digital camera (EXILIM EX-Z4, Casio Computer Co., Ltd., Tokyo, Japan). After wounding, ERI or PS lotion was applied 3 times daily in each group, and the wound was photographed. Based on image data obtained using the digital camera, the wound area was measured using Image J software (Bioarts, Co., Ltd., Fukuoka, Japan). The wound area on each measurement day as a percentage of that on the day of wound induction was calculated, and the healing effects of ERI lotion were evaluated.

### 2.6. Histological examination

In 2 mice, burn wounds on the back were induced by a method similar to the above using an electric soldering iron. One mouse was topically treated with ERI lotion and the other with PS lotion 3 times daily. On day 3 after wound induction, the mice were sacrificed by lethal inhalation anesthesia with diethyl ether. The skin including tissue around the wound was resected and immersed in 20% neutral buffered formalin solution for pathological examination (Kanto Chemical Co., Inc., Tokyo, Japan), and paraffin sections (4 µm in thickness) were prepared and stained with hematoxylin-eosin (HE) to obtain histological cross-sectional specimens of the skin. Subsequently, each specimen was photographed using a digital camera and histologically observed. The preparation and evaluation of histological specimens were outsourced to the Hist Science Laboratory Co., Ltd., Tokyo, Japan.

### 2.7. Observation of blood vessels near the wound

The 2 mice used for histological evaluation were sacrificed on day 3 after burn wound induction. The dorsal skin was resected, and the state of the capillaries in subcutaneous tissue was observed.

### 2.8. Statistical analysis

For each measurement item, the mean and standard deviation of data in each group were calculated. Statistical analysis was performed by employing Wilcoxon's rank sum test, and  $p < 0.05$  was considered significant.

## 3. Results

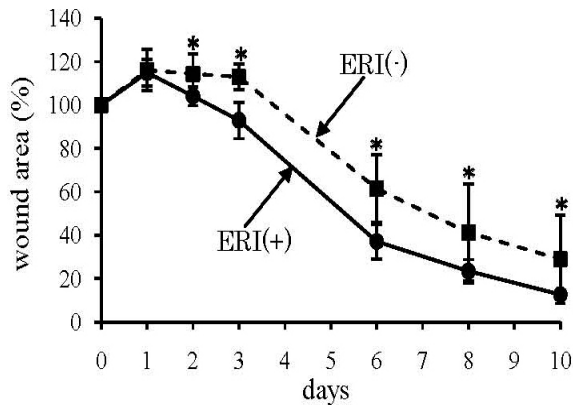
In this study, for the application of ERI to medical drugs, we evaluated the healing effects of ERI lotion

on burn wounds. As general parameters for the evaluation of wound healing, days until the completion of epithelialization and the wound area are used. Therefore, in this experiment, third degree burn wounds were induced on the backs of mice, and the wound area was serially measured and compared with that in the

controls treated with PS lotion.

### 3.1. Time-course changes in the wound area

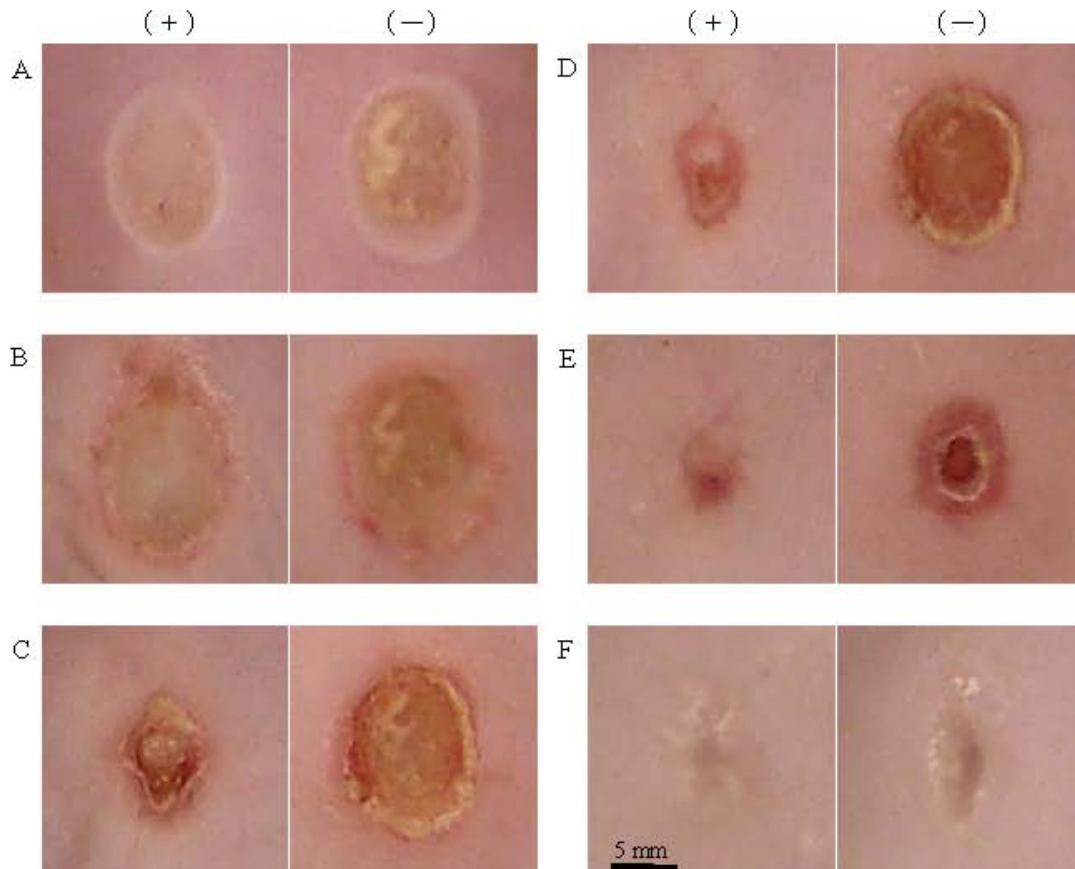
The wound area on each measurement day as a percentage of that on the day of burn wound induction was calculated and compared between the groups (Figure 1). On day 1, the wound area did not differ between the two groups. Wound healing was more advanced in the ERI (+) group compared with the ERI (-) group from day 2, and a definite difference was observed between the two groups from day 3. The wound healing area did not markedly vary among the mice in the ERI (+) group, consistently showing a decrease. In the ERI (-) group, the wound healing area slightly varied on day 6 or more. Wilcoxon's rank sum test of measurement data showed no significant difference on day 1 but significant differences (\*) from day 2.



**Figure 1. Percentage changes in the wound area after burn wound induction.** Serial changes in the wound area on each measurement day as a percentage of that on the day of wound induction (day 0). Data are shown as the mean value  $\pm$  standard errors. ERI (+): ERI lotion application (solid line). ERI (-): PS lotion application (dashed line). In each group,  $n = 6$  (\*:  $p < 0.05$ ).

### 3.2. Changes in healing after burn wound induction

After burn wound induction, ERI or PS lotion was applied 3 times daily in each group, and the burn wound site was photographed on days 0, 3, 6, 8, 10, and 17 and compared between the ERI (+) and (-) groups (Figure 2). As a result, definite healing effects were observed



**Figure 2. Changes in the healing process after burn wound induction.** A: 0 day, B: 3 days, C: 6 days, D: 8 days, E: 10 days, F: 17 days. (+): ERI lotion application, (-): PS lotion application. The central area indicates the wound site.

in the ERI (+) group compared with the ERI (-) group from day 6. On day 10, almost complete healing was observed in the ERI (+) group. A comparison of scars after complete healing (day 17) revealed milder skin depression and less noticeable scars in the ERI (+) than in the ERI (-) group.

### 3.3. Microscopy inside burn wounds

Cross-sections ( $\times 20$ ) of the wound site after HE staining on day 3 are shown in Figure 3. Here, the specimen after treatment with ERI lotion (Figure 3A) is indicated by (+) and that after treatment with PS lotion (Figure 3B) by (-), and a specimen of a normal area without a burn wound as a control (Figure 3C) is also shown. The subcutaneous tissue (SC) and the cutaneous muscle (CM) represent subcutaneous tissue and the cutaneous muscle, respectively.

In the ERI (-) specimen, there were many parts not stained with HE (closed arrowhead) in the SC (Figure 3B), indicating the presence of many interstitial spaces, blood vessels, and lymphatic vessels. In the ERI (+) specimen, the SC was densely stained, suggesting less extensive wounding compared with the ERI (-) specimen

(Figure 3A). However, the ERI (+) specimen showed more unstained parts (open arrowhead) in the CM than the ERI (-) specimen and wide interstitial spaces in the CM, suggesting muscle fiber atrophy (Figure 3A).

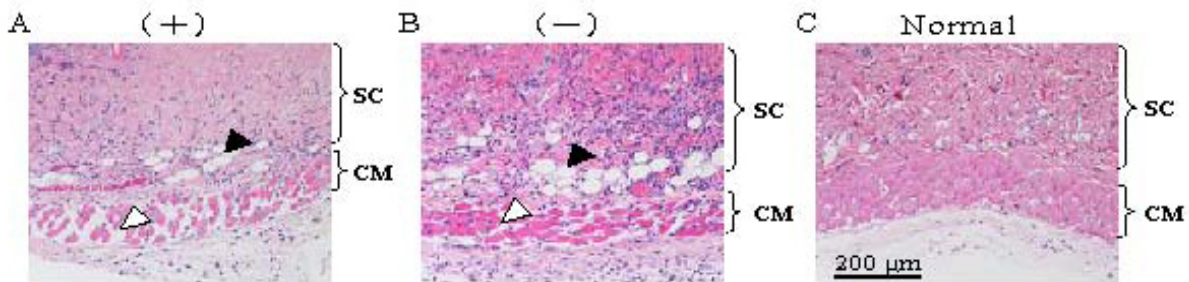
There were few inflammatory cells (mainly neutrophils) stained bluish purple with HE at the normal sites (Figure 3C) but many inflammatory cells in the ERI (+) and (-) specimens. However, inflammatory cell infiltration was more marked in the ERI (-) than in the ERI (+) specimen (Figures 3A and 3B), suggesting less severe wounding in the latter.

### 3.4. Blood vessels in subcutaneous tissue on day 3 after burn wound induction

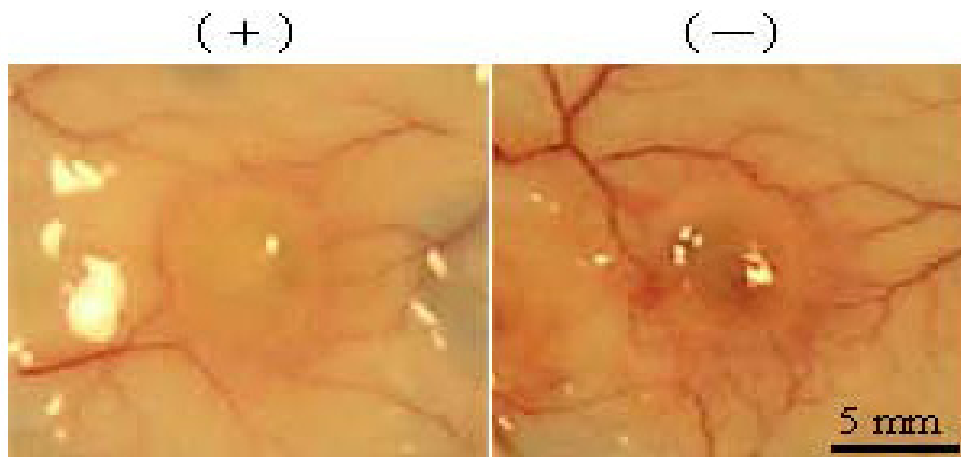
Capillaries near the wound were observed from the subcutaneous tissue side (Figure 4). There were more capillaries around the burn wound in the ERI (-) than in the ERI (+) specimen.

## 4. Discussion

To confirm burn wound healing after ERI application, we evaluated the effects of ERI application on the



**Figure 3. Histological images of the skin after burn wound induction (day 3).** All photographs are HE-stained cross-sectional images of the skin ( $\times 20$  magnification). (+): ERI lotion application (A), (-): PS lotion application (B), Normal site: (C). A and B: Burn wound site, C: Normal site. SC: Subcutaneous tissue, CM: Cutaneous muscle.  $\Delta$ : Parts not stained with HE in the SC,  $\blacktriangle$ : Parts not stained with HE in the CM.



**Figure 4. Status of blood vessels observed from the subcutaneous tissue side on day 3 after burn wound induction.** (+): ERI lotion application, (-): PS lotion application. The central area indicates the wound site.



wound surface. Initially, ERI was directly applied, but it dried immediately, and so a moist environment could not be maintained. Therefore, to maintain a moist environment on the wound surface, we evaluated the use of ERI ointments or dressings. However, to facilitate the observation of serial changes of the wound surface, a lotion was prepared using cmc-Na, which is transparent and viscous and has no pharmacological action. Cmc-Na, which is a polymer for pharmaceutical preparations also used for dressings, is freely soluble in water, viscous, and stable, and serves as a protective colloid, and, therefore, is often used as a dispersal agent or binder. In this experiment, the viscous lotion form was used to retain ERI at the burn wound site for a certain time and maintain its healing effects.

After injury, ERI or PS lotion was applied 3 times/day, and skin tissue in the mouse burn wound model was resected on day 3. For the histopathological examination of the effects of ERI, HE-stained specimens were prepared and observed by microscopy. In specimens after ERI or PS application, third degree burns were histologically observed, but a comparison of histological findings between the two specimens showed definite differences (Figure 4).

In the ERI (-) specimen after treatment with PS lotion as a control, the epidermis in the burn wound area disappeared, and the entire dermis including its residual appendages underwent coagulation-liquefaction necrosis. In addition, necrosis extended to the CM, and muscle fibers were atrophied. A large amount of nuclear disintegration substances (positive for hematoxylin) was sporadically observed (Figure 3B). The SC showed marked inflammatory reactions.

Histological examination of the specimen after treatment with ERI lotion showed coagulation necrosis from the epidermis at the burn wound site to the entire dermis. However, the necrosis was mild compared with the ERI (-) specimen, and the structure of muscle fibers was preserved without the progression of necrosis to liquefaction (Figure 3A). In the SC, nuclear disintegration substances (positive for hematoxylin) were sporadically observed, but inflammatory reactions were mild compared with the ERI (-) specimen.

From Figure 4, blood vessels from the subcutaneous tissue side to the area near the wound were confirmed. On day 3 after injury, capillaries could be confirmed at the site of injury and surrounding area in the ERI (-) specimen but were negligibly observed in the ERI (+) specimen. Considering the presence of a clearly smaller wound area in the ERI (+) than in the ERI (-) specimen on day 3 (Figure 1), this decrease in capillaries in the ERI (+) specimen suggests an early, smooth transition from the inflammation stage to granulation stage on day 3. Altavilla *et al.* reported that the presence of angiogenic factor in the injured area is closely associated with the process of wound healing. They

suggests that a defect in vascular endothelial growth factor (VEGF) regulation might be associated with wound-healing disorders (16). Alternatively, ERI may have a suppressive effect on angiogenesis based on the capillary decrease shown in Figure 4.

Thus, the effects of ERI lotion were evaluated in a mouse skin burn wound model. When the depth of burn wounds is similar among mice, ERI lotion applied to wounds reduces necrotic changes and inflammatory reactions occurring until day 3 after injury.

In general, the wound healing process consists of 3 stages, *i.e.*, inflammation, granulation, and reconstruction stages. Day 3 in this study corresponds to the inflammation stage, when cells such as neutrophils phagocytose injured cells and secrete physiologically active substances such as cytokines. In this study, inflammatory cell infiltration from the dermis to subcutaneous tissue was less pronounced in the ERI (+) than in the ERI (-) specimen, suggesting that ERI reduces inflammation (Figures 3A and 3B).

The burn wound healing process is complex, involving inflammatory aspects, such as cytokines, growth factors and proteases (17,18). Naito *et al.* reported that the beneficial effects of electrolyzed alkaline water (EAW) on aspirin-induced gastric mucosal injury may be attributed to its anti-inflammatory properties *via* inhibition of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) expression (19). Therefore, we considered that ERI inhibits the expressions of factors such as TNF- $\alpha$ , VEGF and matrix metalloproteinases (MMPs). In the future, some additional research may elucidate the mechanism and role of this process.

Histological examination was performed in the inflammation stage until day 3 after injury but not in the granulation or reconstruction stage. In the future, we intend to study the effects of ERI in these stages. In addition, on the wound surface with a maintained moist environment and infection, the control of infection is important, and we also intend to evaluate dressings (such as hydrocolloids, hydropolymers, and alginic acid salts) using ERI with antimicrobial effects.

In this study, the effects of ERI on burn wounds were evaluated, and its wound healing-promoting effects were observed. Although further evaluation is necessary, the clinical application of ERI is expected.

## 5. Conclusion

We experimentally evaluated the healing effects of ERI on third degree burn wounds. The results of this study suggest that ERI is effective for improving burn wounds and promotes burn wound healing.

## Acknowledgements

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**Letter****Compliance with the triage protocols****Jean-Pierre Tourtier<sup>1,\*</sup>, Laurette Mangouka<sup>1</sup>, Stphane de Rudncki<sup>1</sup>, Delphine Lemoullec<sup>2</sup>**<sup>1</sup> Intensive Care Unit, Military Hospital Val de Grce, Paris, France;<sup>2</sup> Psychiatric Departement, Hospital Clermont de l'Oise, Clermont Cedex, France.**Keywords:** Triage, pediatric, compliance

We would like to congratulate Maeda *et al.* for their study, very well designed, concerning the development of the pediatric triage program in Japan (1). Pediatric telephone triage protocols are a complex and challenging issue, and both under triage and over triage need to be evaluated. With the tendency for increased emergency department (ED) use and overcrowding, triage has become a critical step in ED functioning. Cost-containment efforts have focused on prescreening of patients by telephone to determine who may be redirected to a routine visit or given home advice instead of seeking in the ED. The use of a computerized system could theoretically improve triage by improving its completeness and enhance reproducibility. But telephone triage relies on accurate triage tools for identifying major cases and compliance with the triage protocols. We would like to go further into the debate, and highlight that compliance of both parents and physicians with triage protocol is the corner-stone of triage efficiency. The study of Maeda *et al.* used a hypothetical group of children to investigate the effectiveness of the decision analysis model assessed. The rate at which parents follow the recommendations given by physicians during telephone triage was estimated based on another study (2). Parents' compliance toward recommendation about medical attention was hypothesized to 93.5%. This could appear optimistic. For example, Baker *et al.* studied parental compliance to nurses' telephone triage advice. These authors found that parents had a low compliance when advised to bring their children to the ED – only 42% of non-private patients and 46% of private patients complied with the advice (3). Moreover, the telephone

triage pediatrician's compliance with triage protocol itself was not considered by Maeda *et al.* We would only point out that in real life experience, the rate of compliance of triage personnel with guidelines can be low (4). Piccotti *et al.* assessed the percentage of consistency with the triage process drawn up at the level of pediatric ED, and concluded that they were a need for further efforts to improve compliance with the protocol and pursue a higher degree of uniformity in evaluation by triage personnel (5). Moreover, Wachter *et al.* (evaluating the implementation of a set of standardized pediatric telephone triage protocols) have found that 58% of nurses felt confined by the protocols, and 42% admitted intentional deviation from them, when they believed that optimal patient care mandated that they do so (6). Correlation among dispositions determined by triage providers was poor, despite instructions to follow protocols as closely as possible. Although it is a basic assumption that protocols operate by standardization, these results indicate that nurses did not reliably choose the same protocol in a given case and did not reach the same triage endpoint even when they followed the same protocol. As suggested by Poole *et al.*, nurses may decide "under some circumstances to follow their intuition rather than the protocol's recommendation" (7). And it was found that physicians too can easily break from protocols to achieve disposition of patients, especially the more experienced one (8). Although protocols may be useful to help triage, their application must be studied rigorously before they can be safely disseminated for general use, as far as many bias linked with poor compliance can make telephone triage protocols less seducing in practice.

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# BioScience Trends

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193:149-154.

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