Effects of Electrical Stimulation on the Mast Cell of Skin in Rats

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전기자극이 흰쥐의 피부 비만세포에 미치는 영향

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< Abstract >

The purpose of this study was to determine the effect of electrical stimulation on the number of MCs and percent of degranulated MCs in rat skin. Twelve male Sprague-Dawley rats were divided into two group; electrical stimulation group (n=6) and control group (n=6). Each animals hair on the back was removed. The electrical stimulation group received an positive rectangular pulsed electrical stimulation, while the control group was given the same treatment without electricity. The biopsy specimens were fixed in formalin, embedded in paraffin and stained with toluidine blue-nuclear fast red and alcian blue-safranin O, respectively. The MCs were counted using a light microscope and computerized image analysis system and calculated as the density and the percent. A t-test showed a significantly higher density of MCs in the electrical stimulated rats than control rats (p <0.05), and the percent of degranulated MCs in the electrical stimulated rats was higher than in the control rats (p<0.05) in toluidine blue stained sections. The density of MCs was significantly higher in the electrical stimulated rats than the control rats in alcian blue-safranin O stained sections (p<0.01). An analysis of variance showed that the densities of CTMCs was significantly lower than the densities of MMCs and mixed MCs in electrical stimulated rat in alcian blue-safranin O stained sections (p<0.05). These results suggest that the electrical stimulation may have potential for increasing the number of MCs and lead to degranulate the MCs in rat skin.

KEY WORDS : mast cell, skin, electrical stimulation, rat

< 국문초록 >

전기자극이 흰쥐 피부의 비만세포 수 및 탈과립에 미치는 영향을 규명할 목적으로 본 실험을 시행하였다. Sprague-Dawley계 수컷 흰쥐 12마리를 6마리씩 무작위로 전기자극군과 대조군에 배치하고 전기자극군 흰쥐의 등에 양극전극을 부착한 후 맥동빈도 100 pps, 맥동기간 140 μs, 전류량 500 μA로 60분간 전기자극하였으며 대조군은 전극만 부착하고 전류를 통전시키지 않았다. 전자자극 후 등의 피부를 적출하여 포르말린에 고정한 후 파라핀 포매 절편을 제작하여 toluidine blue 및 alcian blue 염색을 하였다. 이 표본을 고배율 현미경하에서 관찰하여 현미경에 부착된 CCD 카메라를 통해 개인용 컴퓨터로 보낸 다음 영상분석 프로그램을 이용하여 비만세포 수, 탈과립 비만세포 수, 비만세포의 아종 수, 부위별 비만세포를 계측한 후 비만세포의 밀도, 탈과립 비만세포 비율, 비만세포 종류의 비율, 분포 비율을 산출하여 분석하였다. 실험 결과 전기자극군의 비만세포 밀도 및 탈과립 비만세포 비율이 대조군보다 통계학적으로 유의하게 높게 나타났으며, 결합조직 비만세포의 비율이 유의하게 낮았다. 또한 비만세포는 표피에는 존재하지 않고 진피에 분포하며 진피 심부에 유의하게 많이 분포하고 있었다. 이러한 결과는 흰쥐 피부의 양극 전기자극이 비만세포의 수를 증가시키고 비만세포의 탈과립을 유발시키고 있음을 시사하고 있다. 향후 비만세포와 관련이 있는 피부질환 및 손상을 대상으로 한 연구가 기대된다.

INTRODUCTION

Mast cell (MCs) was regarded as a kind of a pariah cell, once a day. In recent years MCs have become an increasingly interesting cell. MCs are derived from hematopoietic precusors, and its widely distributed in connective tissue, especially in the skin, the respiratory system, the gastrointestinal and the genitourinary tracts (Galli et al, 1993). In these locations MCs are often adjacent to blood vessels and lymph vessels, epithelial cells, fibroblasts and near or within peripheral nerves (Kitamura, 1989; Galli, 1990; Gottwald et al, 1995).

Skin MCs contain large amounts of bioactive chemical mediators such as chymase, histamine, heparin proteoglycan in their cytoplasmic secretory granules. When the MCs are activated, the MCs resulted in degranulation and chemical mediator release. After degranulation, the degranulated MCs synthesized new granules and transformed to stable MCs through proliferation, maturation and differentiation by MCs proliferative factors such as fibroblast, NGF, IL-3, IL-4, et al (Schwartz and Austen, 1984; Aldenborg and Enerback, 1986; Hebda et al, 1993). Among the chemical mediators, histamine participates in modulation of blood flow and collagen formation, chymase induces an accumulation of collagen fibers by fibroblast proliferation, bFGF activates fibroblasts and mediates angiogenesis, TGF-β participates in proliferation modulation, heparin stimulates the migration of

endothelial cell (Trabucchi et al, 1988; Taipale et al, 1995; Schäffer et al, 1998). These bioactive mediators play a important role in various biological responses such as tissue matrix remodeling in tissue repair, inflammatory skin diseases, blood vessel formation and fibrosis formation (Atkins, 1987; Trabucchi et al. 1988; Tharp, 1987; Rothe et al, 1990; Zhang et al, 1995, Toyoda and Morohashi, 1998). Skin MCs can activate by chemical stimulations such as proteases, chymotrypsin, substance P, infection, allergen. MCs also can degranulate by physical and mechanical stimulations such as contusion, heat, ultraviolet, laser and X-ray (Goetzl et al, 1985; Kitamura, 1989; Galli, 1990; El Sayed and Dyson, 1993a; El Sayed and Dyson, 1996;).

Electrical stimulation for biological growth and repair are well known (Bourguignon and Bourguignon, 1987; Cruz et al, 1989; Biedebach, 1989; Feedar et al, 1991; Agren et al, 1994). Although few reports exist concerning the effect of electrical stimulation on MCs. Reich et al (1991) reported that a positive pulsed electrical stimulation to reduce the number of MCs was observed in a pig's skin wound. Also Taşkan et al (1997) reported that the positive pulsed electrical stimulation decreases the number of MCs and prevents degranulation of MCs in a rat's skin wound. Alterations of MCs number and degranulation of MC with electrical stimulation on normal skin have not been demonstrated. Consequently, the purpose of this study was to ascertain whether electrical stimulation elicits alterations in the number of MC, and the percent of degranulation of MCs

in normal rat skin.

MATERIALS AND METHODS

Animals

Twelve male Sprague-Dawley rats weighing 170-210 g were used. The rats were maintained under clean conventional conditions, under which they were fed standard rat chow (Samyang Feedstuff, Samyang Feedstuffs Co., Ltd., 400-3 Woosan-dong, Wonju, Kangweon, Corea). They had access to tap water ad libitum, and were kept under a 12 h light/dark cycle at a constant temperature of 24±2°C. On the day before treatment, the hair on the upper and lower back were removed using a electric hair clipper (HA 944, Hasung Co., Corea).

Electrical Stimulation

The rats were placed in a clear plastic restrainer and rested comfortably. The shaved upper and lower back area of rat skin were covered with saline-soaked gauze pads (2 x 2 cm), and carbon-silicone rubber electrodes (2 x 2 cm) were placed over each moist gauze, then secured by a restrainer. Electric line cords were inserted through the window in the restrainer. Positive and negative electric line cords connected the electrode on the upper and lower back, respectively. The electrically stimulated rats received electrical stimulation with a current intensity of 500 μA at 100 pps, 140 μs through the electrodes for a duration of 60 minutes using a commercial microcurrent stimulator (Micro Plus^{TM}, BioMedical Life Systems, Inc., 1120 Sycamore Avenue, Suite F, Vista, California 92083, USA). The shape of the applied waveform was monophasic rectangular. Control rats received a sham treatment.

Tissue Sampling and Histochemical Staining

The rats were induced with ethyl ether anesthesia. A 10 x 10 mm sample of the back skin was excised and fixed in 10% phosphate buffered formalin. The tissue sample included subcutaneous tissue and musculature. Following tissue sampling, the rats were sacrificed by ether anesthesia. The fixed tissue sections were dehydrated by ascending graded alcohol series, cleared with xylene using an automatic tissue processor (Citadel 1000, Shandon, Life Sciences International Ltd., Astmoor, Runcorn, England, WA7 1PR). The tissue sample was embedded in paraffin and cut into 5 μm thick serial sections using a rotary microtome (Rotary Microtome HM 340E, Microm Laborger-äte GmbH, Robert-Bosch-Strasse 49, D-6909 Walldorf, Germany). For each rat tissue sample, serial sections were stained with toluidine blue-nuclear fast red (pH 0.5) and alcian blue-safranin O (pH 0.3).

Quantification of MCs

Quantification of MCs and degranulated MCs were performed using a computerized image analysis. For video image analysis, a light microscope (Olympus BX 50, Olympus Optical Co., Ltd., 2-43-2, Hatagaya, Shibuya-Ku, Tokyo, Japan) was linked to a CCD camera (IK-642K Toshiba CCD color camera, Toshiba Co., 1-1-1 Shibatori, Minato-Ku, Tokyo, Japan), and an image processing and analysis system (Image-Pro$^{®}$ Plus, Media Cybernetics, Inc., 8484 Georgia Avenue, Silver Spring, MD 20910, U.S.A.). The software used in this system was a WIN98, along with a computerized image$^{®}$ analysis software Image-Pro$^{®}$ Plus (ver 3.01). Quantification was performed using x40 objectives. At this magnifications, each pixel of the computer image corresponded to 4.128 μm and each field represented an area of 0.018 mm^2 of the tissue. The number of MCs was counted in 120 serial fields from the randomly chosen region of the sample of the rats skin. The total field per sample had an area of 2.16 mm^2. The number of MCs in the full thickness, the superficial and deep half layer of the dermis were counted in toluidine blue-nuclear fast red stained sections and calculated as the density per square millimeter excluding hair follicle and adnexa. The number of degranulated MCs in dermis were counted in toluidine blue-nuclear fast red stained sections and calculated as the percentage. The number of connective tissue MCs (CTMCs), mucosal MCs (MMCs) and mixed MCs in alcian blue-safranin O stained sections were calculated as the percentage.

Data Analysis

For a comparison of the density of MCs and degranulation ratio of MCs between the electrical stimulation and the control groups, a Student's *t*-test was used. the Student's *t*-test was also used to determine a difference of MCs distribution between the deep dermal and the superficial dermal layers. The distribution of MCs subtypes was analyzed by analysis of variance. If this analysis showed statistical significance, the Duncan multiple range test was applied *post hoc*. The statistical interpretation was based on a 0.05 significance test level. SPSS WIN (ver 7.5) software was used for the analyses .

RESULTS

Histological Findings

In the toluidine blue-nuclear fast red stained section, the MCs generally appeared stained deep blue. The intact MCs maintained the integration of the cell outline, but the degranulated MCs disrupted the cell outline partially or totally and showed a discharge of granules (Fig 1.). The MCs were absent at the epidermis, but abundant at the dermal layer. The majority of MCs were located around blood vessels near the boundary of panniculus carnosum (Fig 2.). Three types of MCs was observed in alcian blue-safranin O stained sections. CTMCs showed red staining, MMCs showed blue staining. while mixed type MCs revealed red and blue staining (Fig 3.).

Density of MCs

The MCs density in the dermal layer of the electrical stimulated and the control rats are shown in Fig 4. Thus, the density of MCs in toluidine blue-nuclear fast red and alcian blue-safranin O stained sections of electrical stimulated rats was higher than that of the control rats (p <0.05, p<0.01, respectively). The Density of the MCs in the toluidine blue-nuclear fast red stained section was lower than that in alcian blue-safranin O stained section, but not significantly different.

Degranulation of MC
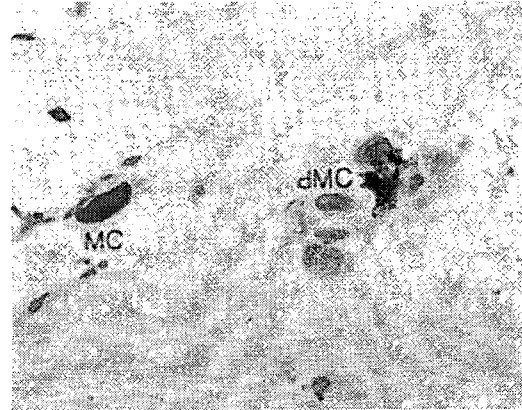
The ratio of degranulated MCs in toluidine blue-

Fig 1. The MCs show deep blue staining. The intact MCs maintained the integration of the cell outline (MC), while the degranulated MCs disrupted the cell outline (dMC). Toluidine blue-nuclear fast red, x400.
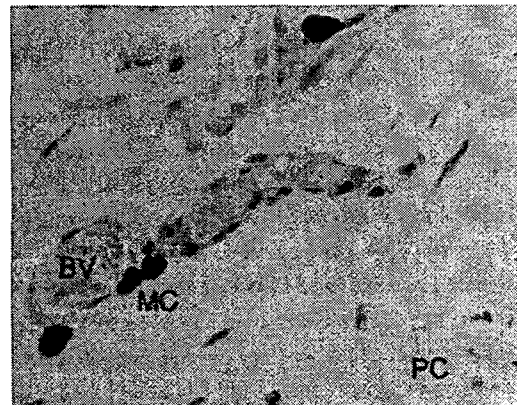

Fig 2. The MCs are evident around blood vessels (BV) near the boundary of panniculus carnosum (PC) in deep dermis. Toluidine blue-nuclear fast red, x400.

nuclear fast red stained sections of electrical stimulated and control rats are given in Fig 5. The percent of degranulated MCs of electrical stimulated rats was significantly higher than that of the control rats (p<0.05).

Percent of MC Subtypes

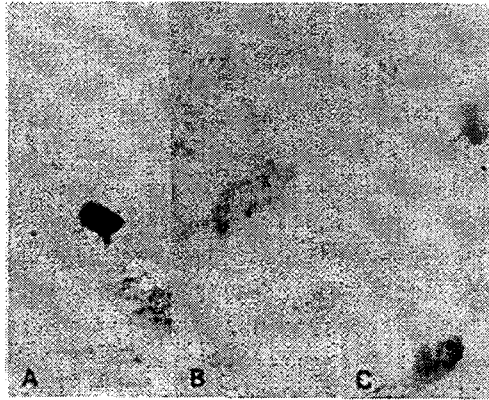The percent of three types of MCs of electrical stimulated and control rats are given in Fig 6. The

Fig 3. The red stained MCs are CTMCs (A), red and blue stained MCs are mixed type MCs (B), blue stained MCs are MMCs (C). Alcian blue-safranin O, x400.

CTMCs were significantly lower than the MMCs and mixed MCs in electrical stimulated rats (p<0.5).

Distribution of MC

The ratio of MCs in deep dermal and superficial dermal layer of electrical stimulated and control rats are given in Fig 7. The MCs in the deep dermal layer were significantly greater than that in the superficial dermal layer in electrical stimulated and control rats (p<0.05), but there was no significant difference between electrical stimulated and control rats.

DISCUSSION

In this experiment, the density of MCs in the electrical stimulated rats was significantly higher than the density of MCs in the control rats. The percentage of degranulated MCs was significantly higher in the electrical stimulated rats than in the control rats in toluidine blue-nuclear fast red stained sections. In the alcian blue-safranin O stained section, we regard the red stained MCs as matured stable CTMCs, the blue stained MCs as MMCs and the blue-red stained MCs as mixed MCs. The percentage of CTMCs was significantly lower than the percentage of MMCs and mixed MCs in the electrical stimulated rats compared to the control rats. In addition, the number of MCs in the deep dermal layer was higher than that in the superficial dermal layers in
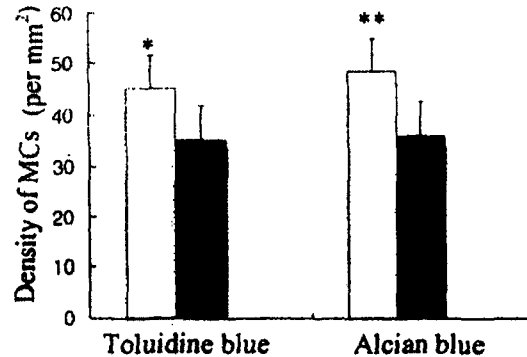

Fig 4. Comparison of MCs density in the dermis of electrical stimulated (open bar) and control (closed bar) rats. MCs density in toluidine blue-nuclear fast red stained, and alcian blue-safranin O stained sections of the electrical stimulated rats was significantly higher than in the control rats (p<0.05, p<0.01, respectively).
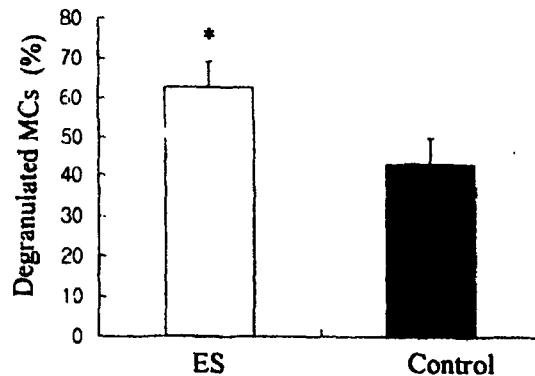

Fig 5. Comparison of the percentage of degranulated MCs in the dermis of the electrical stimulated (ES) and the control rats. The percentage of granulated MCs was calculated in toluidine blue-nuclear fast red stained sections. The percentage of degranulated MCs in the electrical stimulated rats was significantly higher than in the control rats (p<0.05).

the electrical stumulated and the control rats. These findings are similar to the results that were reported by El-Sayed and Dyson (1993b).

These results showed that electrical stimulation increases the total number of MCs and the number of degranulated MCs in rat skin. However, Reich et al (991)
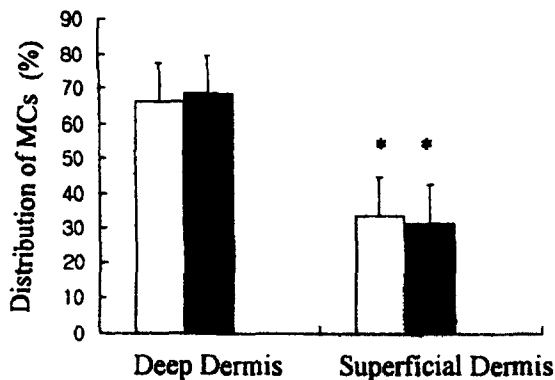
Fig 6. Comparison of the MCs distribution in the deep and superficial dermal layer of electrical stimulated (open bar) and control (closed bar) rats. The MCs in deep dermal layer was significantly higher than superficial dermal layer in both the elelctrical stimulated and the control rats (p <0.05).
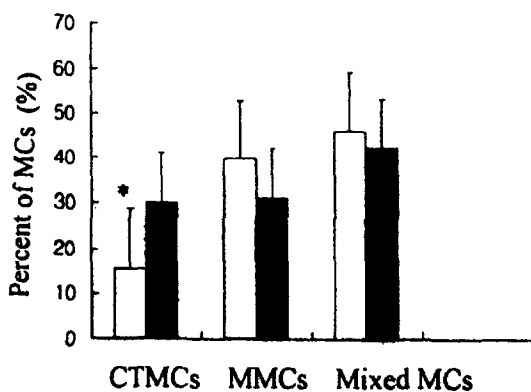

Fig 7. Comparison of the MCs subtypes distribution in alclan blue stained sections of electrical stimulated (open bar) and control (closed bar) rats. The CTMCs was significantly lower than mixed MCs and MMCs in electrical stimulated rats (p<0.05).

reported that positive pulsed electrical stimulation reduced the number of MCs. Tasşkan et al (1997), also reported that positive pulsed electrical stimulation decreased the number of MCs and prevented degranulation of MCs in skin. Our results seems to disagree with these reports. This discrepancy may result from the difference of experimental material. Their studies were conducted on wounded skin, but we performed the study on normal rat skin.

The mechanism for the alteration of MCs by electrical stimulation in normal skin is not known. We suppose that the effects of electrical stimulation on MCs are possible based on a review of scientific reports. First, electrical stimulation of skin may stimulate the nerve fibers in dermis, activating the MCs, while initiating degranulate and chemical mediator releases. The skin MCs are usually located around blood vessels. They are also found around nerve fibers anatomically. The nerve cell excitation caused degranulation of MCs and chemical mediator release (Goetzl et al, 1985). Electrical stimulation influence to the voltage-gated ion channels in nerve cell membranes in dermis, leads to excitation of the nerve, directly. The second possible explanation for the effect of electrical stimulation on MCs is related to changes in the concentration of ions. MCs are known to be sensitive to fluctuation of positive ions. For example, high concentration of Ca^{++} in MCs are known to induce the degranulation and proliferation of MCs (Yurt, 1981; Tharp, 1987). Electrical stimulation activate the voltage-dependent Ca^{++} channels in MC, and leads Ca^{++} influx into MCs.

Further investigations with many groups of animals and various parameters of electrical stimulation should be performed to substantiate the optimal dosage of electrical stimulation for affect to MCs.

CONCLUSION

The purpose of this study was to examine the effect of positive electrical stimulation for alterations in the density of MCs and percentage of degranulation of MCs in normal rat skin. The results of this study revealed a statistically significant higher level of MCs density and degranulated MCs ratio in electrical stimulated rats compared to that in control rats. The CTMCs were significantly lower than the density of the MMCs and mixed MCs in the electrical stimulated rats. This result suggest that positive electrical stimulation may have potential for increasing the number of MCs and leading to degranulating the MCs in rat skin.

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