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Effect of taurine on wound healing

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Summary. Taurine which has antioxidant effects is also known to have effects on cell proliferation, inflammation and collagenogenesis. The aim of this study was to investigate the effect of taurine on incisional skin wounds.

The mice incised on the dorsal area were divided into control and experimental groups. Saline was injected intraperitoneally to half of the animals in the control group and locally applied to the other half. Fifty mM taurine solution was given intraperitoneally to the first half of the experimental animals and locally to the second half of the experimental group.

After four days of treatment, malondialdehyde (MDA) and histamine levels as well as the tensile strength of the wound tissue were measured. Structural alterations in epidermis and dermis were histologically evaluated.

The locally administreated taurine significantly increased wound tensile strength by decreasing the MDA and histamine levels and prevented the degranulation of the mast cells. These observations suggest that taurine may be useful on wound healing.

Keywords: Amino acids – Taurine – Histamine – Malondialdehyde – Tensile strength – Mast cell – Wound healing

Introduction

Taurine (2-aminoethane sulfonic acid), an amino acid containing sulphur, is found in almost all tissues in mammals, and it is considered that taurine plays various important physiological roles in each organ. For example, it has been demonstrated that taurine might have a modulating action on the function of the central nervous system (Kuriyama, 1980) and a membrane stabilizing action on the sarcoplasmic reticulum (Huxtable and Bressler, 1973). It has been reported that taurine enhances cell proliferation (Hunter, 1989) and viability (Pasantes-Morales et al., 1984) and that this affects inflammation and collagenogenesis (Gordon et al., 1986; Wang et al., 1989). On the other hand,

it has been shown that taurine possesses antioxidant effects (Alvarez et al., 1983; Pasantes-Morales and Cruz, 1985) and/or that this suppresses lipid peroxidation (Nakashima et al., 1983).

Lipid peroxidation is a process in which the unsaturated fatty acids of the cell membrane react with free radicals and cause oxidative damage (Ginsberg et al., 1988; Basaga, 1990). Recently, there has been an increasing interest in the actions of free radicals, especially because they are known to be involved in tissue damage (Basaga, 1990). For example, some compounds, such as histamine, which destroy membrane fluidity and integrity may be released by free radicals (Mannaioni et al., 1988). The effects of the free radicals on wound healing have been investigated and clarified (White and Heckler, 1990). These studies suggest that free radicals play an important role in collagen damage and in the early decrease of wound margin strength.

Some antioxidants have been used in order to remove the negative effects of oxygen free radicals on wound healing (Högstrom, 1987; Bergren et al., 1988; Foschi et al., 1988). Exogenous taurine has been administreated to prevent oxidant damage in many tissues, but it has not been investigated on incisional skin wounds.

The aim of this study was to determine the role of taurine on incisional skin wounds by investigating the effect of taurine on lipid peroxide formation (MDA), histamine levels, wound tensile strength, and histological structure.

Materials and methods

This study was performed on 26 Swiss Albino mice of both sexes, weighing 32.2 ± 1.0 g, They received water and food ad libitum before the operations.

The animals were anesthetized with ether and their backs were shaved and cleaned with tincture of iodine. Two standard wounds, (4 cm long and of full-thickness skin) were incised on both sides at the spine (parallel to each other and one cm from the spine). The wounds were sutured by 3 silk stitches (5-0). After surgery, each animal was placed in an individual cage.

The mice were divided into two groups; each group was further divided into two subgroups.

I. Control groups: Sterile isotonic saline was applied to fourteen mice over the post-operative period.

Intraperitoneal saline group ($S_{I.p.}$): Saline (60 μl) injection was intraperitoneally applied to seven mice.

Local saline group (S_L): Saline was locally applied to the wounds (30 μl to each wound) of the other seven mice.

II. Experimental groups: Taurine was freshly dissolved in sterile isotonic saline prior to injection or local application (50 mM aqueous solution)

Intraperitoneal taurine group ($T_{I.p.}$): Taurine solution (60 μl) was intraperitoneally applied to six mice.

Local taurine group (T_L): Taurine solution was locally applied to the wounds (30 μl to each wound) of the other six mice.

All treatments, starting on day zero and continuing to day five, were applied twice a day for four days. On day five, animals were killed by ether anesthesia. The scars and surrounding skin were excised. Malondialdehyde and histamine contents and tensile strength of wound tissues were measured. Structural alterations in epidermis and dermis were investigated histologically.

Measurement of wound tensile strength

Full thickness skin flaps of 5 mm^2 were dissected for mechanical testing. The wound was located in the middle of the flap. The skin was squeezeed with the clamps exactly 1 mm away from and parallel to the wound, giving a total distance of 2 mm between the clamps and the wound at the center. The tensile strength was determined by a Grass Model 7 Polygraph (Grass force displacement transducer FT 03) (Çelebi et al., 1994).

Malondialdehyde content of the skin

Malondialdehyde content of the skin was estimated by the method of Uchiyama and Mihara (Uchiyama and Mihara, 1978).

Histamine content of the skin

Histamine content of the skin was measured using the method reported by Shore et al. (Shore et al., 1959).

Histology

Skin tissues were cut into small pieces, fixed in % 2.5 phosphate buffered glutaraldehyde for 2 hour and then post fixed in 0.1 g osmium tetroxide, dehydrated in serial alcohol and embedded in araldite. The thick sections of 0.5 μm were then stained with toluidine blue and examined with an Olympus BH2 light microscope.

Statistics

All values were expressed as mean $\pm$ SE. The significances of the differences among various treatments were analyzed by using Student's t test. $P < 0.05$ was considered as significant.

Results

The MDA and histamine levels present in the wound tissue and wound tensile strength are shown in Table 1. The significances of the differences among various treatments are shown in Table 2.

The application of both saline and taurine locally or intraperitoneally did not bring about a significant difference in the MDA levels of the wound tissues ($S_L - S_{I.p.}$: $p > 0.5$, $T_L - T_{I.p.}$: $p > 0.05$).

However, it was observed that the application of taurine in both ways was effective in the increase of wound tensile strength. In those treated with taurine locally, it was established that MDA and histamine levels were the lowest and that wound tensile strength was the highest. On the contrary, in those treated with i.p. saline, the wound tensile strength was the lowest and the MDA and histamine levels were the highest. In this group, the wound histamine level was higher by % 75 when compared with that of the local saline group (Table 1). However, since in the i.p. saline group the standard

Table 1. MDA and histamine levels and tensile strength of the skin wounds

	Control		Taurine	
	Local (S_L)	Intraperitoneal ($S_{I.p.}$)	Local (T_L)	Intraperitoneal ($T_{I.p.}$)
MDA (nmol/g)	$\bar{X} \pm SE$ 61.9 ± 4.3 $n = 7$	$\bar{X} \pm SE$ 58.9 ± 3.5 $n = 7$	$\bar{X} \pm SE$ 35.8 ± 2.9 $n = 6$	$\bar{X} \pm SE$ 43.9 ± 5.8 $n = 6$
Histamine (γ/g)	$\bar{X} \pm SE$ 4.6 ± 0.4 $n = 6$	$\bar{X} \pm SE$ 8 ± 2.2 $n = 5$	$\bar{X} \pm SE$ 2.4 ± 0.4 $n = 5$	$\bar{X} \pm SE$ 7.4 ± 0.8 $n = 6$
Tensile Strength ($N \cdot cm^{-1}$)	$\bar{X} \pm SE$ 0.4 ± 0.01 $n = 7$	$\bar{X} \pm SE$ 0.2 ± 0.01 $n = 5$	$\bar{X} \pm SE$ 0.7 ± 0.04 $n = 5$	$\bar{X} \pm SE$ 0.5 ± 0.06 $n = 5$

Table 2. The significances of the differences between groups (p values)

	MDA	Histamine	Tensile strength
S_L-$S_{I.p.}$	$p > 0.5$	$p > 0.05$	$p < 0.01$
S_L-T_L	$p < 0.001$	$p < 0.01$	$p < 0.001$
T_L-$T_{I.p.}$	$p > 0.05$	$p < 0.001$	$p < 0.05$
$T_{I.p.}$-$S_{I.p.}$	$p < 0.05$	$p > 0.05$	$p < 0.01$

error was large, the difference between these two groups was not found to be statistically significant.

Histological findings

Local saline group: It has been observed that the flat laminated epithelium was highly developed and the dermis was normal. In dermis, generally, mast cells with big and little granules was noted. Few granules were dispersed amongst collagenous bundles (Figs. 1, 2).

Intraperitoneal saline group: In this group, the epidermis consisted of irregular 3 or 4 layers and none of the layers of the flat laminated epithelium was completely developed. It was determined that the cytoplasm of the newly developed cell was very intensive. Moreover, the continuation of the inflammatory cell infiltration in the dermis was determined. As these cells were in large number, the collagenous fibers were observed in partly intensive thin bundles and they were few. In these preparations most of the mast cells were partially degranulated (Figs. 3, 4).

Local taurine group: In this group, the flat laminated epithelium consisting of young cells was highly developed. In the dermis the connective tissue fibers and the cells were normal. Mast cells with intensive granules filling the cytoplasm were also seen (Figs. 5, 6).

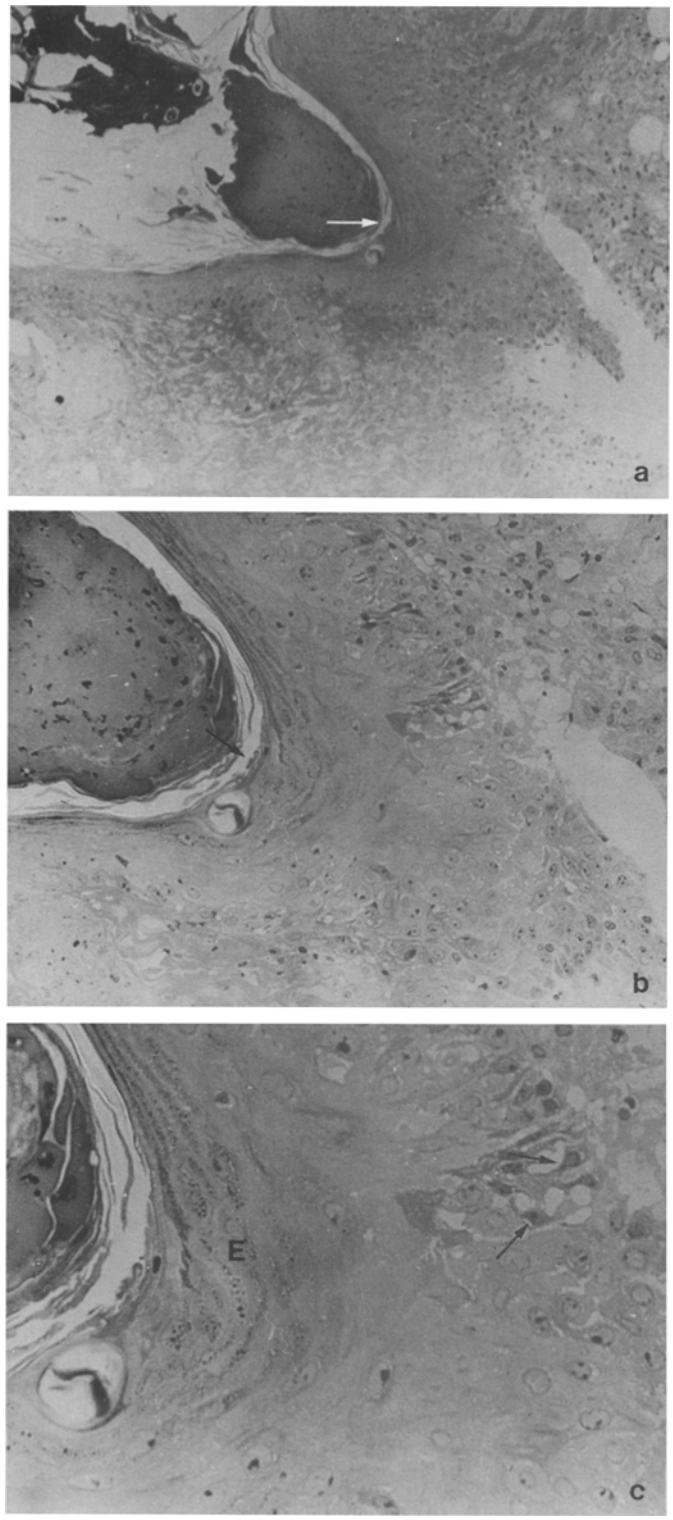

Fig. 1. a Local saline group; wound area (arrow). Toluidine Blue ×100. **b** Local saline group; wound area (arrow). Toluidine Blue ×200. **c** Local saline group; epidermis (*E*) atipic cells in basal (arrows). Toluidine Blue ×400

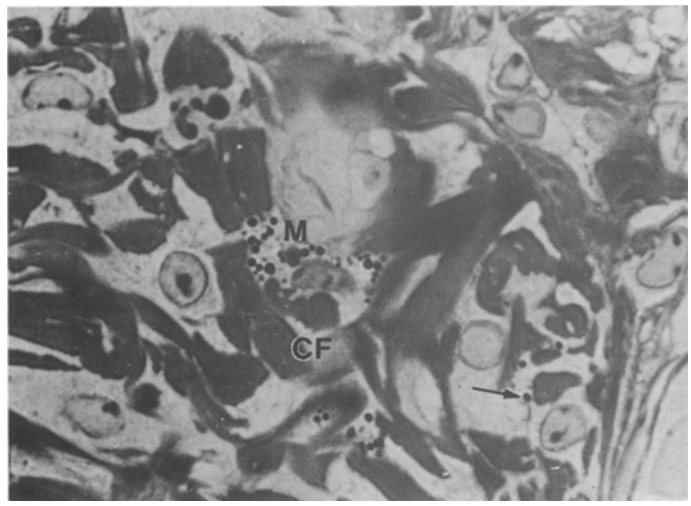

Fig. 2. Local saline group; plane of section is deep portion of wound area. Collagenous fibers (*CF*), partially degranulated mast cell (*M*) free granules in intercellular area (arrow). Toluidine Blue ×1000

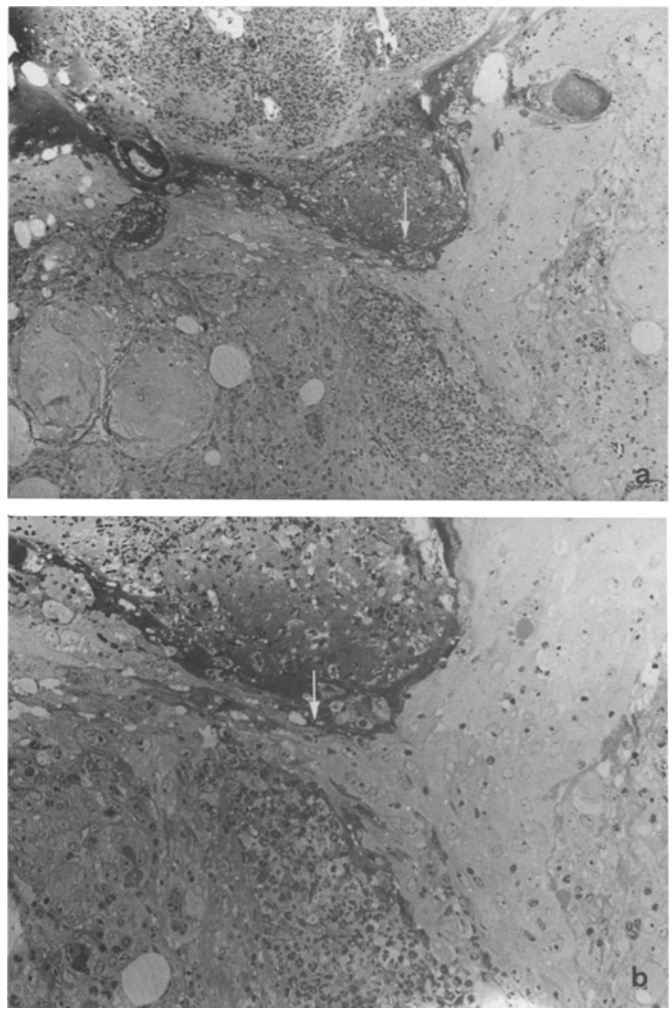

Fig. 3. **a** Intraperitoneal saline group; wound area (arrow). Toluidine Blue ×100. **b** Intraperitoneal saline group; wound area (arrow). Toluidine Blue ×200. **c** Intra-peritoneal saline group; epidermis (*E*), Inflammated cells (arrow), crust (*C*). Toluidine Blue ×400

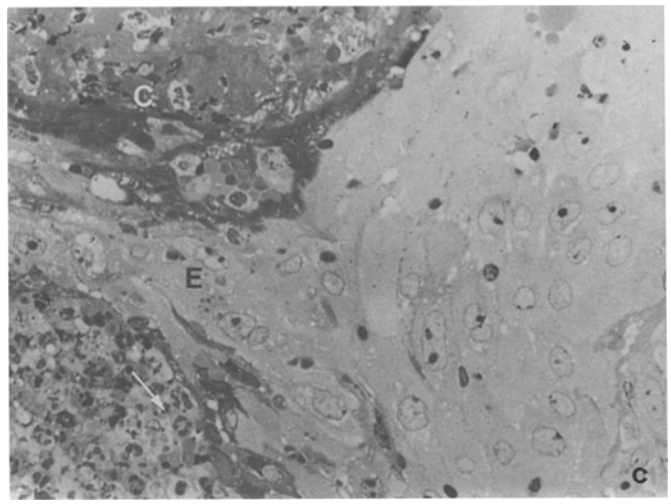

Fig. 3c

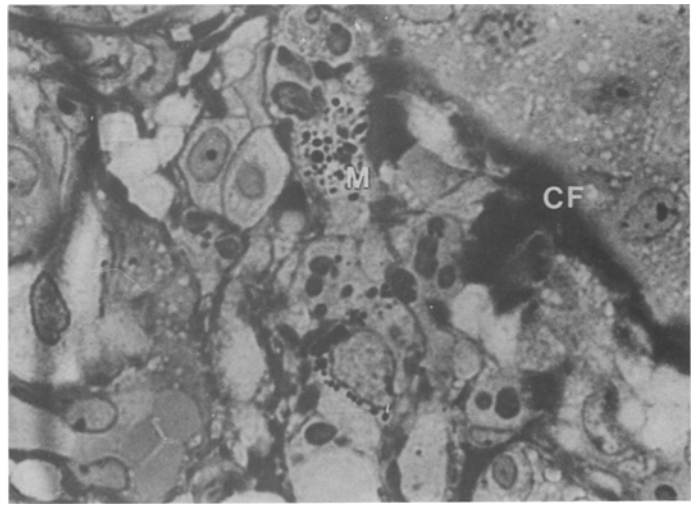

Fig. 4. Intraperitoneal saline group; deep portion of wound area, few collagenous fibers (*CF*) partially degranulated mast cell (*M*). Toluidine Blue ×1000

Intraperitoneal taurine group: The epithelization was not appeared. In dermis, the collagenous fiber and inflammated cells were observed. Mast cell granules were seen irregularly in intertitiel area. On the other hand, possibly, a mast cell with its big size and characteristic nuclear form which discharged content of granules was observed with its emtied granule sites. (Figs. 7, 8).

Discussion

It is known that water homeostasis and tissue hydration is effectively regulate and facilitate the substance movements in the interstitial area in wound heal-

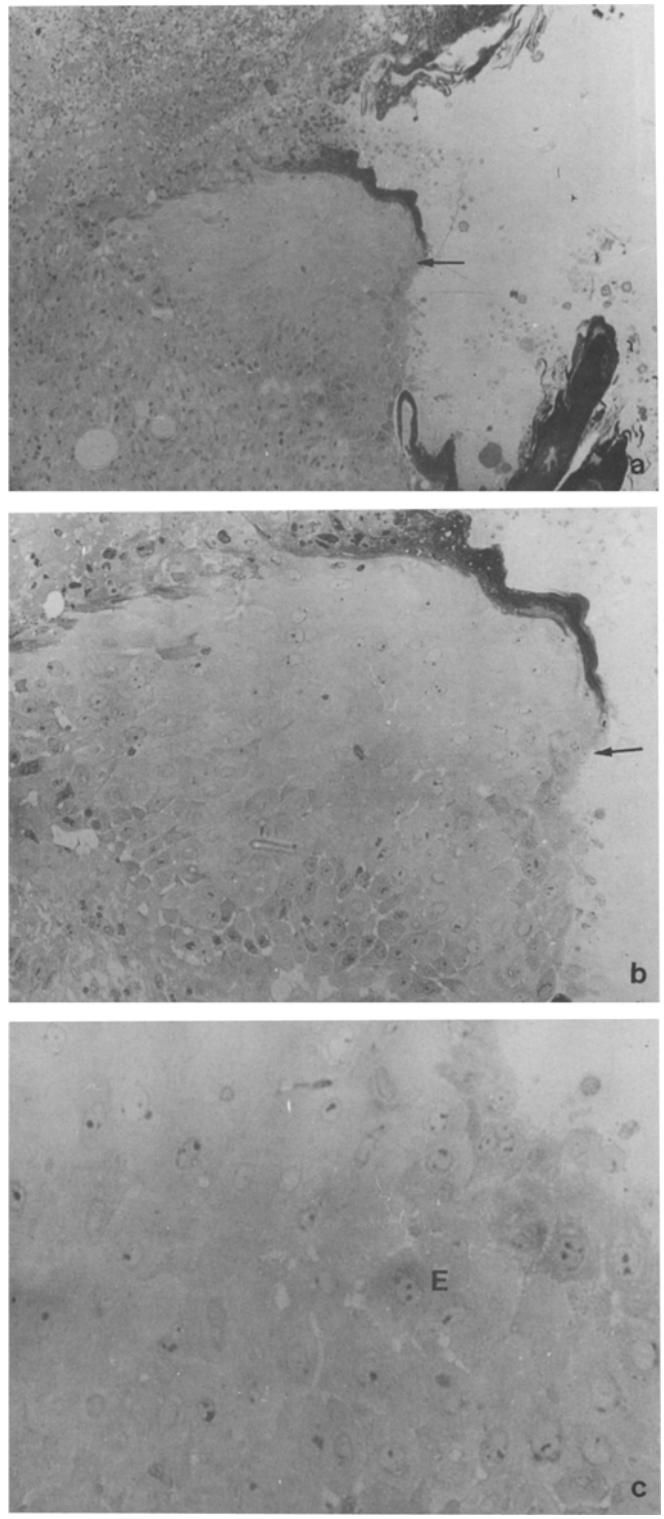

Fig. 5. **a** Local taurine group; wound area (arrow). Toluidine Blue ×100. **b** Local taurine group; wound area (arrow). Toluidine Blue ×200. **c** Local taurine group; epidermis (*E*), Toluidine Blue ×400

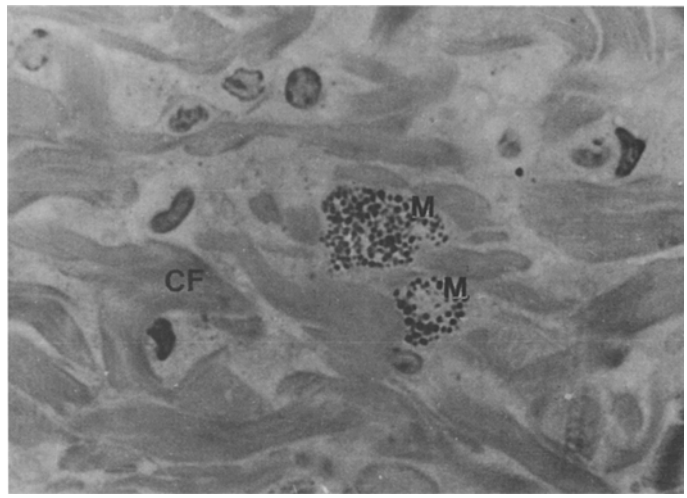

Fig. 6. Local taurine group; a view of dermis, collagenous fiber (*CF*), mast cell cytoplasm completely filled with granules (*M*). Toluidine Blue ×1000

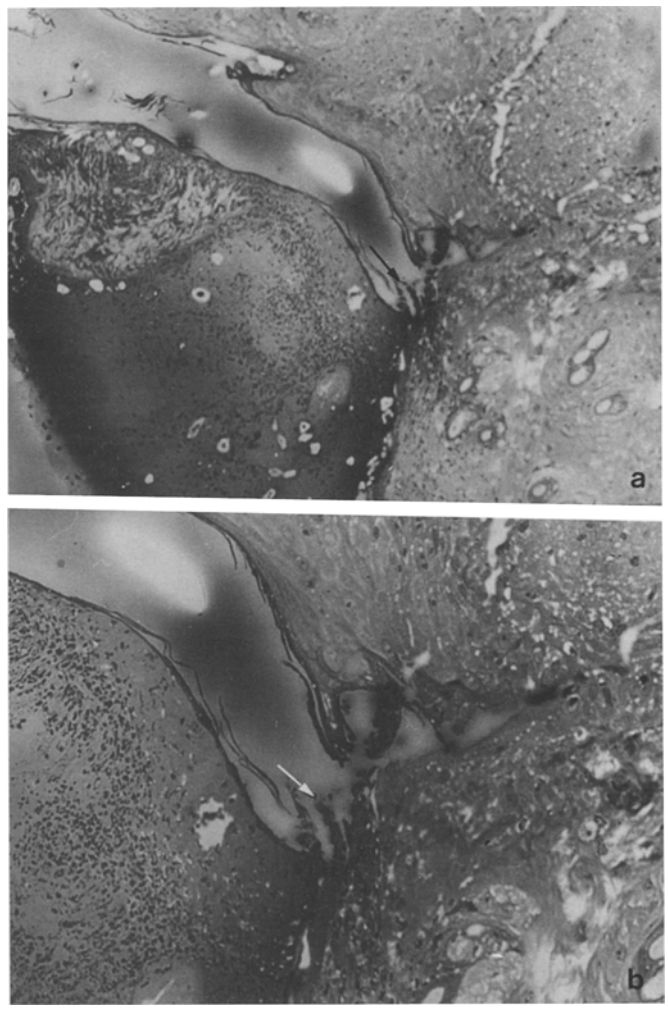

Fig. 7. **a** Intraperitoneal taurine group; wound area (arrow). Toluidine Blue ×100. **b** Intraperitoneal taurine group; wound area (arrow). Toluidine Blue ×200. **c** Intraperitoneal taurine group; epidermis (*E*), inflammated cells (arrow). Toluidine Blue ×400

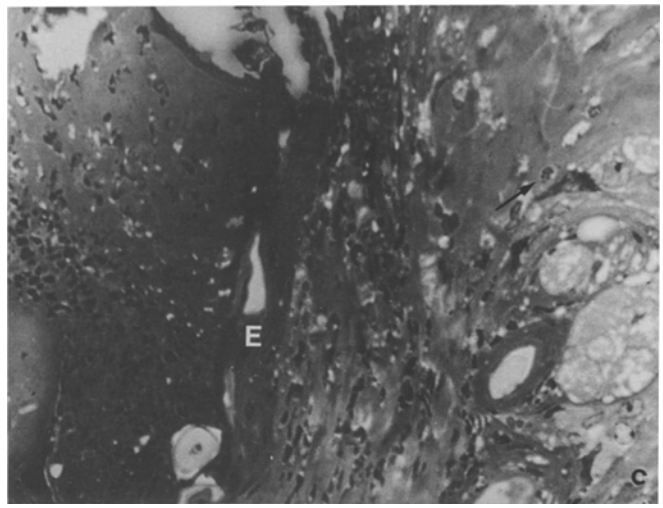

Fig. 7c

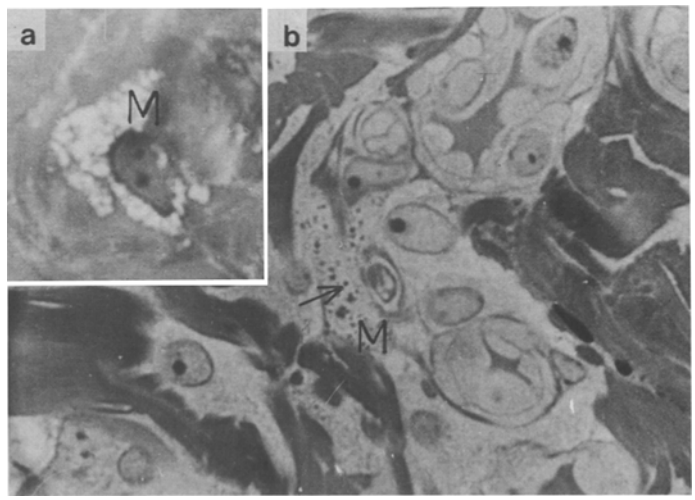

Fig. 8. Intraperitoneal taurine group. **a** Without granules mast cell (*M*). Toluidine Blue ×1000. **b** Partly degranulated mast cell (*M*). In this cell cytoplasm, the granule whice has an intensive colored middle area and a light colored peripheric area was appeared (arrow). Toluidine Blue ×1000

ing (King et al., 1991; Jeffrey, 1992). In our study, aqueous medium in wound area would be the result of the local administrations of saline and taurine plus saline. And then some substances like as histamine will be diluted. So aqueous medium will be the concequence of local administration of solutions compare to i.p. administrations.

Increased tensile strength and decreased histamine levels were determined in local groups compared to i.p. groups. These results supported the King and Jeffrey's findings.

On the other hand, isolated rat serosal mast cells exposed to free radical generating systems, increased the release of histamine in accordance with the MDA production (Januszkiewicz and Faiman, 1984; Faiman, 1986). Meanwhile the secretory granules of rat mast cells have been shown to be one of the subcellular sources of superoxide production (Henderson and Koliner, 1978).

In our study, the effect of taurine on lipid peroxidation in wound tissue was determined with the MDA production and it was found that taurine decreased the MDA levels. This finding is consistent with previous studies, in which the taurine was shown to decrease the MDA levels or in the case of taurine deficiency there is a significant increase in MDA levels (Nakashima et al., 1982; Pasantes-Morales et al., 1985; Harada et al., 1990). Decreased histamine and MDA levels in taurine administreated groups according to those the saline groups, may be the concequence of the effect of taurine on the inhibition of lipid peroxidation in mast cells and other cells.

In literature it was reported that the increased in ion and water permeability due to the membrane damage caused by lipid peroxidation (Pasantes-Morales and Cruz, 1985) was prevented by taurine probably with a calcium dependent mechanism (Harada et al., 1990) and that by this way it stabilized the membrane (Huxtable, 1992).

We observed that the histamine levels in the i.p. groups were higher than those of the local groups. In addition, the mast cells were fewer and degranulated. The reason for this may be the insufficient concentration of the i.p. taurine in the wound tissue and/or the stress of injections. In the case of stress, cathecolamines are released by the sympathetic stimulation and that these amines may cause the release of histamine from mast cells (Mannaioni et al., 1988). On the other hand, it was observed that i.p. administration of taurine effectively elevated the circulating taurine levels, but a similar increase did not occur during inflammation (Wang et al., 1989). This fact was related to the oxidation of taurine by peroxidases released from the inflamated cells. In our study, we can state that taurine levels may be oxidised similarly in blood, and as a consequence of this, taurine levels decreased. The locally administreated taurine may have reached the high concentrations in the wound area so, it has been found more efficient.

There are some studies on the regulating role of taurine on collagenogenesis and on the prevention of abnormal collagen production such as fibrosis (Gordon et al., 1986; Wang et al., 1989).

In our study, tensile strength and histological findings supported that local taurine was more effective in collagen production.

On the other hand, the role of histamine on collagen formation has been reported in some studies. While Kahlson et al., (1960) reported the increased wound tear strength, Sandberg (1962) and Fitzpatrick and Fisher (1982) reported the decreased collagen formation as well as wound tear strength by increased histamine levels. There are also some data which indicate that histamine stimulates collagen biosynthesis at low doses and inhibits at high doses (Dabrowski and Maslinski, 1981).

In our study, it has been observed that the effects of histamine on wound healing may also be related to the levels of MDA in wound tissue. In the circumstances where both histamine and MDA were high (such as $S_{I.p.}$ group), it has been established that tensile strength was extremely decreased. When MDA levels were high and histamine levels were low or viceversa (such as in groups S_L and $T_{I.p.}$), tensile strength has been found greater compared to that of $S_{I.p.}$ group. As shown in local taurine treated group where the histamine and MDA levels were low, it has been observed that tensile strength was at the highest value in all studied groups.

In conclusion, we may state that locally administreated taurine decreased the MDA and histamine levels in wound tissue and increased the tensile strength and accelerated the wound healing. Therefore, it can be assumed that taurine plays an important role in wound healing. However, further studies are required to explain the above mentioned effects of this amino acid.

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