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Full Length Research Paper

Both omega-3 and omega-6 polyunsaturated fatty acids stimulate foot wound healing in chronic diabetic rat

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The potential role of omega-3 (ω-3) and omega-6 (ω-6) fatty acids on wound healing in chronic diabetic diseases is of interest and controversial. In this experimental study, the effect of topical application of fish and corn oils containing ω-3 and ω-6 fatty acids on skin wound healing in chronic diabetic rat has been evaluated. Rats were randomly divided into five groups (n = 7). First group was served as normal or control group. In diabetic groups, one group was non-treated group (shame group) and two groups received fish and corn oil (FO-group and CO-group), respectively. The last diabetic group was treated with both fish and corn oil (FCO-group). Treatment was done from 4 weeks after the induction of diabetes till complete wound healing. All animals were wounded by a 2 cm^2 incision in their dorsum. Wound surface area and required time for full healing were measured at various post-operated periods. The histological characteristics were studied by using hematoxilin and Eosin (H & E) method. Our results showed that surface area of wound in FCO-group was lesser than that non-treated group at 11th, 15th and 20th post-operative days significantly. Moreover the percentage of the wound healing in FCO-treated and non-treated groups was 98 and 70% at the 20th day, respectively. Histological studies showed that epidermal growth, cellular diffusion, density of collagen in FCO-group approximately were the same as control group. Topical application of fish and corn oil together may result in an acceleration of skin wound healing in chronic diabetic rats.

Key words: Fish oil, corn oil, wound healing, chronic diabetes.

INTRODUCTION

The incidence of foot amputation in diabetic individuals is increasing in recent years and has been reported as 0.5 to 5 in 1000 diabetic persons (Jeffcoate and Harding, 2003). Majority of diabetic persons are aware from this matter that the pathogenesis of their foot ulceration is complex and may cause the amputation of their foot. In studies of causal pathways leading to lower-extremity diabetic amputation, foot ulcers preceded ~84% of the amputations (Reiber et al., 1999).

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Different steps of wound healing process, such as co-agulation, inflammation, proliferation and epithelialisation may be affected by diabetes. Diabetic foot amputation could be reduced by prevention of deficiency in wound healing as a main factor causing foot ulcer (Yue et al., 1986). This deficiency could be as a result of increase in blood sugar and it is followed by consequences, such as dysfunction of white blood cells, thickness of base membrane of capillary cells, decrease of flow blood, hypoxia, etc. (Stepanovic et al., 2003).

Using of endogenous and exogenous bioactive molecules may be one of the suggested ways to control the deficiency. Endogenous and exogenous bioactive molecules may be acquired from an everyday typical diet

and can themselves be potent mediator and/or regulators of many cellular processes (Ruthig and Meckling-Gill, 1999). Among different type of dietary fat, fatty acids are such bioactive molecules and using these molecules may be one of the suggested ways to control the deficiency. The purpose of this study was to evaluate the effects of topical application of fish and corn oils containing ω-3 and ω-6 fatty acids on skin wound healing in chronic diabetic rat.

We also used two oils with together in mentioned process and their effects were studied.

MATERIALS AND METHODS

Animals

Eight week-old Sprague Dawley male rats (200 to 250 g) were used. Animals received standard pellet food and water *ad libitum*. The temperature in the animal room and examination room was maintained at 23 ± 2°C with a relative humidity of 55 ± 5%. Light was on a 12:12 h light-dark cycle. During the study, four animals were housed in a transparent rectangular cage. The experimental protocol was approved by the University Animal Care Committee.

Experimental groups

Rats were randomly divided into five groups (n = 7) as follow: normal or control group, diabetic group without treatment (shame group), treated diabetic group with fish oil (FO-group), treated diabetic group with corn oil (CO-group), treated diabetic group with fish and corn oils (FCO-group).

Induction of diabetes

Experimental diabetes was induced in diabetic groups by subcutaneous injection of 50 mg/kg Streptozotocin (STZ) between two ears. Control animals received saline alone. To control the hypoglycemia shock and death of diabetic rats, animal were feed by decreasing sugar solution for 3 days. Diabetes was verified 5 days later by estimating hyperglycemia using glucometer (Glucoplus, Canada). Glucoplus strips are impregnated with glucosoxidase enzyme. Only animals with non-fasting blood glucose levels greater than 300 mg/dl were considered diabetic (the normal blood glucose level is 90 to 110 mg/dl in rat). Diabetic and normal rats were maintained for 8 weeks after induction of diabetes (Waisundara et al., 2008; Sukumar et al., 1997). Then, the middle dorsal skin were shaved and wounded by a vertical 4 cm incision in aseptic condition (Chithra et al., 1998).

Treatment

Fish and corn oil were purchased from Sigma Company (menhaden, Sigma Co. Germany). Fish and corn oils were applied to the wounds by using dropper so that all the area of the wounds was covered with oils or saline. Treatments were done as the same time and person, once daily from 8th week after the induction of diabetes till complete wound healing. Wound surface area was measured at various post-operated days, that is, 3rd, 7, 11, 15 and 20th days by using the Fergusson and Logan method. Briefly, a transparency was placed directly over the ulcer, and the wound margins were traced with an indelible pen. These tracings were placed on graph paper, the small squares were counted, and the area of the ulcers was calculated in square millimeters (Griffin et al., 1993). The percentage of wound healing was calculated as follows:

$$\text{Percentage of wound healing} = \frac{\text{Wounds surface at X day - Wounds surface at first day}}{\text{Wounds surface at first day}}$$

Required time for full healing also was measured and at the same time, the histological characteristics were qualitatively studied by using hematoxilin-eosine staining.

Statistical analysis

Differences between groups were compared by using an analysis of variance (one way ANOVA). Tukey-Kramer test was employed afterward, if required. All data are presented as mean ± SD. Differences were considered significant at $P \leq 0.05$.

RESULTS

Our results showed that wound surface area were the same as at first day in all groups, but the parameter decreased in control group from 3rd day to the end of study significantly so that the differences at the initial days were low and at the end days were high. Wound surface area decreased significantly in FCO-group as compared to other three diabetic groups from 11th to the end of the study. Highest of difference between shame and FCO-groups was seen at 11 and 15th days ($P \leq 0.001$) (Table 1).

The percentages of wound healing significantly increased from 3rd day in control group and from 11th day in FCO-group in comparison to diabetic groups. The maximum of difference concerning percentage of wound healing was seen between FCO-groups and shame groups at 11 and 15th day. However the calculated differences between control and other groups were more than others. In other words, in control group healing process was better in comparison to all diabetic groups and differences were more significant.

In control group, mean required time for full healing was 21.66 days and changed between 22 to 30.33 days in FCO and shame groups, respectively. The maximum differences were seen between shame and control groups and shame and FCO-groups ($P \leq 0.001$).

Histological studies showed that epidermal growth, cellular diffusion, density of collagen in FCO-group approximately was the same as control group. In shame group, the cellular layers of epidermis were less than other groups at 15 and 20th day, but in the FCO-group the thickness of epidermis was normal and about 5 to 8 cellular layers were seen at the 20th day (Figure 2). There was a cellular diffusion in some places of the connective tissue of dermis from 3rd to 20th in shame group, between 3rd to 11th in CO and FO-groups, respectively. This characteristic was rarely seen in FCO-group and control group.

DISCUSSION

The results were according to previous studies that wound

Table 1. Wound surface area in experimental and control group at various post-operated days.

Groups / Days	Control	Shame	Corn oil	Fish oil	Fish-corn oils
3rd	296 ± 5.43	304 ± 2.16[a] 0.001	306 ± 2.8[a] 0.000 0.333	303 ± 5.86[a] 0.007 0.479	303 ± 1[a] 0.002 0.751
7th	265 ± 4.1	279 ± 7.1[a] 0.001	279 ± 8.1[a] 0.003 0.817	276 ± 5.2[a] 0.016 0.332	272 ± 7.5 0.076 0.074
11th	8 2± 4.1	146 ± 4.04[a] 0.000	140 ± 2.04[a] 0.000 0.057	131 ± 4.78[a,b] 0.000 0.007	121 ± 2.12[a,b] 0.000 0.000
15th	34 ± 6.8	97 ± 3.67[a] 0.000	91 ± 5.93[a] 0.000 0.141	89 ± 3.29[a] 0.000 0.64	68 ± 2.28[a,b] 0.001 0.000
20th	13 ± 3.59	55 ± 2.41[a] 0.000	51 ± 1.41[a] 0.000 0.189	49 ± 1.5[a] 0.004 0.074	46 ± 2.3[a,b] 0.000 0.001

[a]Significant differences between control group and experimental groups. [b]Significant differences between diabetic treatment experimental groups and shame group. Values represent mean ± SD. The amount of p_{value} is shown in each cell of table.

wound healing delay in diabetic rats, because two parameters, that is, wound surface area and the percentage of wound healing changed in diabetic groups in comparison to control group significantly (Table 1 and Figure 1). The present study, also demonstrates that fish and corn oils together accelerate wound healing in chronic diabetic rats. Using from the omega-3 and omega-6 contained in fish and corn oil, respectively is controversial. In recent years, the beneficial effects of consumption of fish foods have been showed, so the majority of scientists focus on fish oils to heal ulcer in diabetes. It is also demonstrated that fish oils have anti-inflammatory effects with potential beneficial clinical applications. Albina et al. (1993) illustrated that dietary consumption of a diet rich in omega-3 fatty acids may conspire against the quality of wounds by altering the fibroplastic or maturational phases of the healing response. Hammes et al. (1996) tested whether a 6-month administration of fish oil could inhibit the development of experimental retinopathy of the streptozotocin-diabetic rat and concluded that dietary fish oil supplementation may be harmful for the diabetic microvasculature in the retina.

It is reported that fish oil rich from omega-3 may had positive influences on wound healing by different ways such as: omega-3 fatty acids could enhanced ligament fibroblast collagen formation in association with changes in interleukin-6 production (Hankenson et al., 2000), fish oils may augment endothelium-dependent relaxations, principally by improving the release of nitric oxide from injured endothelium (McVeigh et al., 1993) and omega-3 may change lipid metabolism in ulcer region by leptin gene expression (Hynes and Jones, 2001), etc.

Advantage effects of omega-6 contained in corn oil is also reported in literature. The corn oil may influence the ulcer in normal and diabetic condition by: change in eicosanoids production, especially prostaglandins (PGE2) leukotrienes, lipoxins, etc., inhibition cyclooxygenase (Futagami et al., 2002), increasing of growth hormone secretion from anterior lobe of pituitary gland by derived eicosanoids from omega-6 fatty acids (Giron et al., 1999), prevention from diminish of arachidonic acids in plasma and membrane phospholipids in diabetes, mitogenic effects (Giron et al., 1999; Malasanos and Stacpoole, 1991) and increase of collagens formation (Gembal et al., 1994).

There is a hypothesis that fatty acids may accelerate wound healing by epithelialisation and neovascularisation in diabetic animals. Although, in our study, the growth of epidermis in FCO-group was better than shame group (Figure 1), but it seem that condensation of vessels were similar in all diabetic groups, so it is unknown that the cell division and formation of new vessels processes have an important role on the skin repair. Galjour et al. (2005) reported that combination of demineralized bone matrix proteins and tobramycin are better to heal fracture of femurs than the aforementioned matters alone. They used the tobramycin as antibiotic to control inflammation

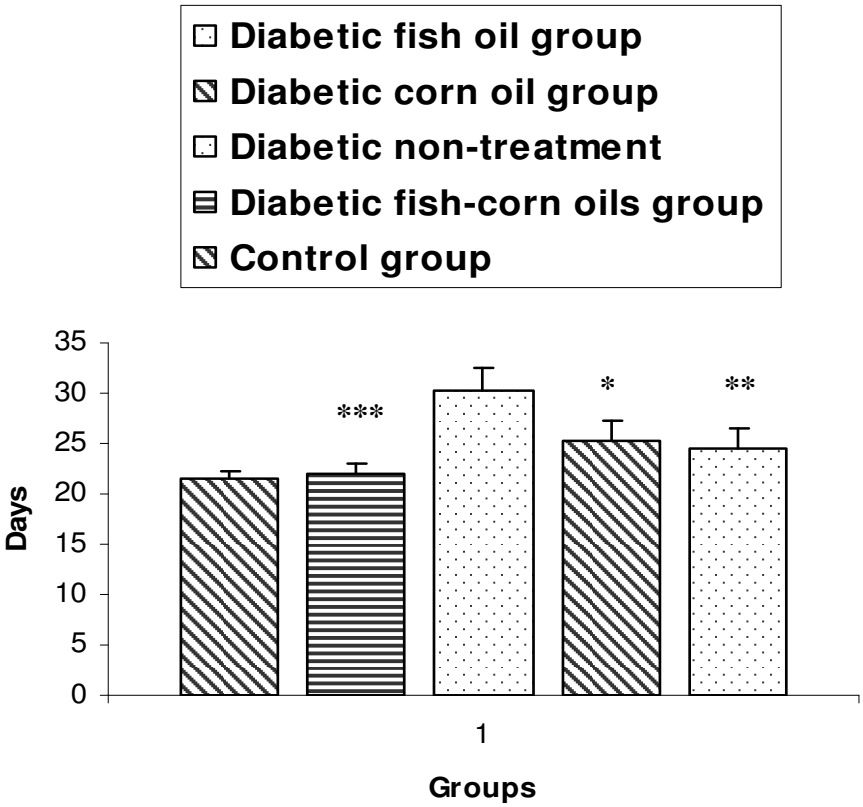

Figure 1. Required time for full healing in all groups. Significant differences between shame and corn oil groups (P ≤ 0.05). **Significant differences between shame and fish oil groups (P ≤ 0.01). ***Significant differences between shame and fish-corn oils groups (P ≤ 0.001). Fish and corn oil separately reduced the time significantly (P ≤ 0.01 and P ≤ 0.05, respectively), but the effect of two oils was more than one alone (P ≤ 0.001). Note to the required time for full healing in control group and compare it with other groups.

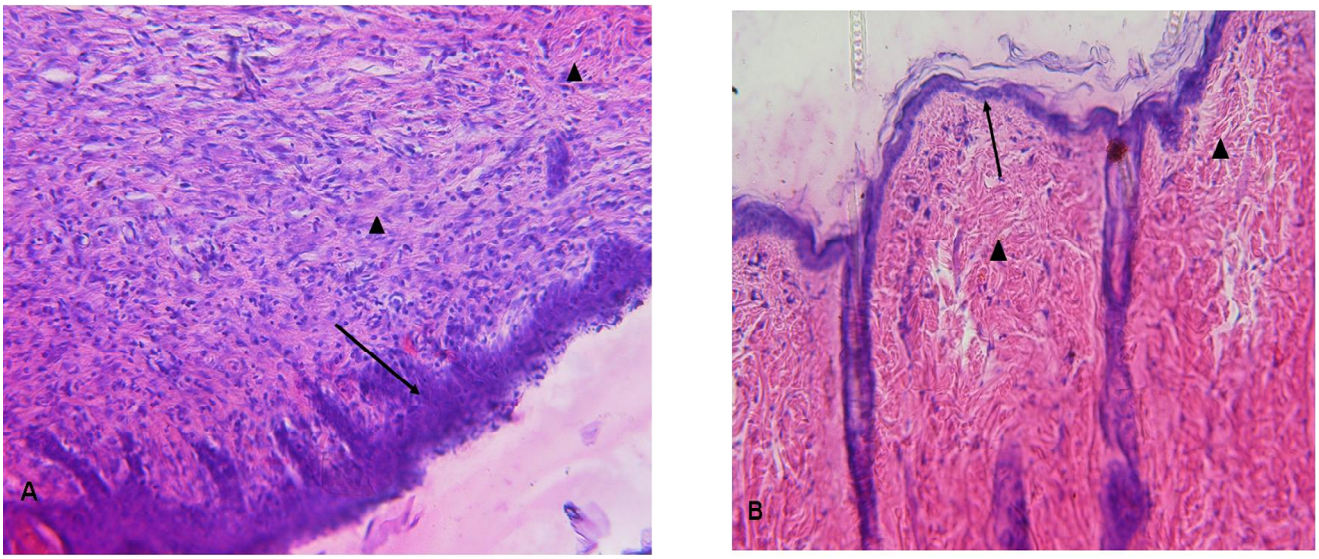

Figure 2. Sections of rat skin stained with hematoxilin-eosine at 20th day. Left panel (a) FCO-group and right panel (b) shame group. Epidermis (arrow) and dermis with collagen fibers (arrowhead). Note to the thickness of epidermis in FCO-group in comparison with shame group. Magnification 400.

result from growth of microorganisms. In our study, the seen cell diffusion in diabetic animals especially in shame group may result from inflammation. It seems that using of both omega-3 and omega-6 together could control the cell diffusion and inflammation mainly at the end days of our experience.

The idea of the present study emerges from a mistake during colleagues' experimental research, such that they examined the effects of fish and corn oil on ulcer in normal rats. During his study a mistake was took place and they gave two oils to a group and saw that wound healing was accelerated in animals.

Conclusion

These results show that synchronous using of both omega-3 and omega-6 fatty acids may be better than omega-3 or omega-6 alone to accelerate wound healing in diabetic rats. It is suggested that more studies will be done on this matter.

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