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Effect of Topical Atropine on Wound Contraction in Swiss Albino Mice : A preliminary report

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Summary

The influence of topical application of atropine ointment was studied in artificially wounded Swiss albino mice. Full thickness excision wounds were made surgically on the dorsum of mice. One mm thick layer of atropine ointment was applied topically on the whole area of wound daily, till the healing of wound. The topical application of atropine ointment significantly delayed the contraction of wound in the atropine treated group when compared with the untreated controls. There was a significant delay in the mean wound healing time (HTM) in the atropine treated group when compared to untreated control group.

Introduction

In the repair of an open wound many fibroblasts in the granulation tissue acquire morphological and biochemical features of smooth muscles and are known as myofibroblasts¹. These myofibroblasts are responsible for wound contraction². Wound contraction is helpful in reducing size of the wound and therefore, in healing of the wound. In burn patients, wound contractions may lead to joint contractures, ectropion of lids and lips, and deformity of various structures. To prevent contracture cumbersome splints are required for many months. It would be ideal if any drug is discovered which can prevent the wound contraction or at least minimize the contracture and prolonged period of immobilization is significantly reduced. In the present paper effect of atropine which produces relaxation of smooth muscles due to muscarinic antagonist effect on the wound contraction in Swiss albino mice has been studied.

Methods

Six to eight week old Swiss albino mice of either sex weighing 35-40 g were selected from an inbred colony maintained under controlled conditions of temperature ($23 \pm 2^\circ\text{C}$), humidity (50 ± 5 percent) and light (10 and 14 hours of light and dark respectively). The animals had free access to sterile food prepared in the laboratory (wheat 70 percent, Bengal gram 20 percent, fish meal 5 percent, yeast powder 4 percent, sesame oil 0.75 percent and shark liver oil 0.25 percent) and water. Five animals were housed in a sterile polypropylene cage containing sterile paddy husk (produced locally) as bedding material.

Production of full thickness skin wound

The animals were anaesthetized using 10mg/Kg body weight of Ketamine. The hairs of the dorsal surface of the animals were removed using an

electric clipper. The cleared surface of the skin was marked by methylene blue using a rectangular stainless steel stencil (25x15mm). The full thickness skin was excised along the markings using toothed forceps, a no. 15 surgical blade and fine pointed scissors. The animals were divided into the following groups :

1) Control Group

Sterilized paraffin was applied over the wound of these animals till the closure of the wound.

2) Atropine group

The excision wounds of these animals were applied with 1 mm thick layer of atropine (10 mg atropine sulphate/g of ointment with sterilized paraffin base) daily in such a way that whole area of the wound was fully covered. The atropine was applied till the complete healing of the wound.

Ten animals were used in each group.

Measurement of the wound area

The length and width of the wound was measured using a vernier caliper. The first measurement was carried out just after wound production and was considered zero day. Subsequent daily measurements were carried out until the wound healed.

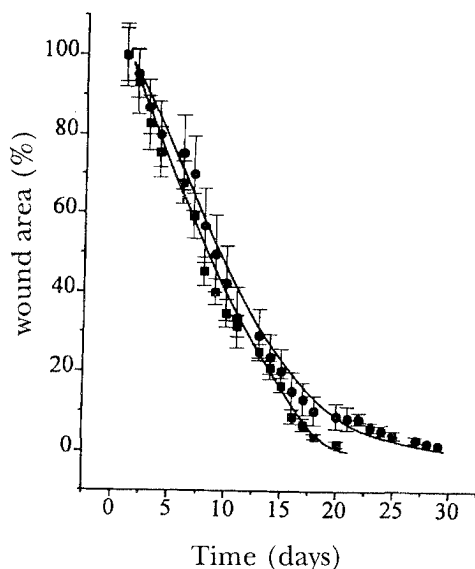


Fig 1. Effect of topical application of atropine on contraction of full thickness wound of mice
 ■ control group ● atropine group

Results

The area of the wound on day zero was considered to be 100% in both the control and atropine treated group and the percentage of wound contraction was calculated from the subsequent measurements (Fig 1).

The control group showed a gradual contraction of the wound and the wound area reduced to approximately 50% by the day 7 post-wounding. By the end of 19 days 50 percent of the animals showed complete wound healing. The mean wound healing time (HTM) of this group was 20.2+0.58 days (Fig 2).

The wound contraction was delayed in the atropine group. In this group wound area reduced to approximately 50 percent by the end of 9 days. By day 20, the wound contraction was significantly delayed in atropine group compared with the control group (P<0.04). Fifty percent of the animals showed complete healing on day 22 post treatment. The mean wound healing time (HTM) in atropine group was 25+1.50 days. The application of atropine significantly delayed the mean wound healing time when compared to the control (p<0.01).

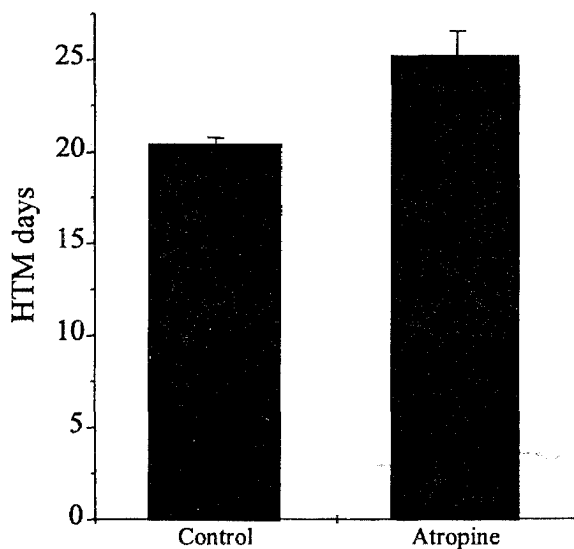


Fig 2. Effect of atropine treatment on the mean wound healing time of artificially wounded mice

Discussion

Wound contraction on the flexor aspect of joint poses a difficult problem to the burn surgeons. Most reliable methods of preventing contractures are application of pressure, cumbersome splinting and physiotherapy. Atropine is the best known muscarinic receptor antagonist³ and it produces smooth muscle relaxation. In clinical practice atropine is used to dilate the pupil (mydriasis), paralyze accommodation (cycloplegia) and as antispasmodic agent. Topical application of atropine ointment significantly delayed the wound contraction by day 20 and the mean healing time (HTM) of the wound increased. Our findings that topical application of atropine delays the contraction of wound may have potential for clinical application in various clinical conditions where delay in wound contraction is to the advantage of patients. Gabbiani¹ et al, in an electron microscopic study of fibroblasts in contracting granulation tissue, have shown that many of the fibroblasts in granulation tissue develop characteristics intermediate between those of typical fibroblasts and those of smooth muscle cells. Majno⁴ et al have tested the contractility of strips of granulation tissue from Sale's "granuloma pouch" like a smooth muscle and reported that 5-hydroxy-tryptamine (5-HT), bradykinin, angiotensin, vasopressin, epinephrine and norepinephrine causes contraction of these strips of granuloma pouch while papaverine caused a slow relaxation. However acetyl choline, tryptophan and histidine had no detectable effect on strips of granuloma pouch. They concluded that fibroblast under certain conditions are capable of modulating toward a cell type that is structurally and functionally similar to smooth muscle and named these cells as myofibroblasts. Myofibroblasts are considered responsible for granulation tissue contraction².

The literature on modulation of wound contraction by various medication⁵⁻⁷ and irradiation^{8,9} is scanty. Study of biochemical and biophysical parameter of wound in rats has been shown to accelerate wound contraction on application of honey⁵ arginine (aminoacid)⁶. Calcitonin gene-related peptide (CGRP) has shown to modify the

wound healing in the rats by increasing the rate of contraction⁷. The wound contraction has been reported to be significantly delayed in the denervated area in rats¹⁰. Experimental studies¹¹ have shown that CGRP and other neural factors are supplied by axonal transport from the neuron cell bodies and are concentrated in nerve terminals. This may explain the delayed contraction in denervated area because cutting a nerve obviously prevents the supply and transport of CGRP into the wounded area.

The glycosaminoglycan chain of decorin and decorin core protein has been shown to delay the contraction of cultured human skin fibroblasts embedded in a three dimensional collagen lattice¹². Using cultured fibroblasts rich in f-actin bundles, Clark¹³ et al have demonstrated that the platelet and monocyte isoforms of platelet derived growth factor (PDGF; AB and BB) can stimulate fibroblast to contract collagen gel matrix in a time course similar to that of wound contraction.

It has been reported that fibroblastic precursors and fibroblasts require support from the bone marrow during wound healing¹⁴. Hence low dose radiation to whole body which results in bone marrow depression results in diminished wound contraction and wound healing^{8,9}. Since the dermal fibroblasts are relatively resistant to irradiation, high doses of local skin irradiation is needed to impair wound healing⁸.

From the present investigation it is apparent that delay in wound contraction by the topical application of atropine may help to minimize solve the problem of wound contraction significantly, if application is continued till apoptosis (programmed cell death) occurs. Desmouliere¹⁵ et al have reported that in late phases of wound healing L-smooth muscle actin positive myofibroblasts (which are responsible for wound contraction) show signs of apoptosis (programmed cell death) and finally disappear. Topical atropine application will be particularly significant over areas like eyelids and lips where designing and maintaining the splints for prevention of contractures is a difficult task. However it is a preliminary report and further detailed exploration is required be-

fore reaching a definite conclusion. In the present preliminary study no attempt was made to assess the contribution of epithelisation in wound healing. However, it does not seem to influence conclusion as there is no report of atropine influencing epithelisation. We presume same amount of epithelisation occurs in both atropine treated and control group. The side effects of atropine due to absorption of topical atropine from larger area remains a possibility in a clinical setting.

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