EFFECT OF PENTAZOCINE ON WOUND HEALING.

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Summary

Apart from several factors, certain drugs are also known to influence the wound healing process. Exploration of drug influence on healing appears to be everlasting, since new drugs are added quite frequently to the existing list. The information on healing of some drugs routinely used particularly during perisurgical period in clinical practice is also poorly documented. Therefore present study was conducted to explore the effect of pentazocine, a commonly used post-operative analgesic, on wound healing.

Pentazocine significantly suppressed healing of incision wounds and dead space wounds as observed by decreased breaking strength of 10 day old incision wounds and granulation tissue as well as granuloma dry weight without any significant effect on healing of excision wound. The mean scar area in pentazocine group did not significantly differ from the corresponding control values. There was no significant difference in number of days required for complete epithelisation. Microscopy of haematoxylin and eosin stained sections of granulation tissue revealed markedly decreased population of fibroblasts and collagen deposition in pentazocine group in contrast to control animals. The present study shows that pentazocine adversely affect incision wound healing. If the present results were to be extrapolated to clinical situation, pentazocine as a post operative analgesic would increase wound complications. Clinical studies in this regard are worthwhile.

Key words: Dead space wounds, Excision wounds, Healing, Incision wounds, Pentazocine.

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Introduction

Wound healing, the most common problem faced in clinical practice is extensively and repeatedly subjected to investigation with an idea of developing new techniques to prevent faulty or delayed healing and to discover ways of accelerating the healing process. Highly dynamic and complex process of healing essentially involves cell proliferation to restore anatomic and functional continuity. Therefore, antiproliferative drugs could be expected to retard healing and some of them have been reported to retard the healing process [1,2]. Despite a great deal of controversy in this regard, cytotoxic drugs like 5-fluorouracil when used post-operatively was reported to increase wound complications in patients with breast tumors [3]. Pentazocine a commonly used analgesic has been reported to possess cytotoxic effect on Ehrilch ascitis tumor cells in vitro [4].If so it could be expected to interefere with healing and therefore, it was felt desirable to explore its effect on wound healing. Though, the drug is commonly used as post-operative analgesic neither its influence on healing of surgical wounds nor in experimental wounds has been documented. In the present study pentazocine was investigated for its effect on different wound models viz., excision, resutured incision and dead space wounds.

Materials and methods

Animals: Male Wistar rats, weighing between 150-200g were starved overnight with free access to water prior to wounding. Under light halothane anaesthesia, each animal was inflicted with one type of wound has described below under aseptic conditions.

Drugs used and their doses: Pentazocine lactate (Fortwin brand) purchased from local market was diluted with distilled water to get the desired concentration. Taking adult dose as 30mg, equivalent dose for rats (2.70 mg/kg) was calculated with the help of table divised by Paget and Barnes[5].Animals in each wound groups were divided in to control (n=6) and treated group(n=6).The treated animals received pentazocine 2.70 mg/kg (in 0.25 ml) intraperitoneally once daily for 3 days starting from wounding day, while controls received equivalent amount of saline i.p. The drug was given for 3 consecutive days to simulate post operative administration of pentazocine by some surgeons in clinical practice.

Excision (open) wounds:

A circular, full thickness skin of 2.5 cm diameter was excised away from the dorsum of the neck after clipping the hair, to get wound area of 500 mm^2 (490 mm^2) as described by Morten and Melone [6].The wound healing was monitored by noting reduction in wound area on 4,6,8,10, 12 days till epithelisation was complete by tracing the raw wound area on graph paper. On complete epithelisation (as indicated by denuded scars without any raw area), scar was traced on graph paper, shape was noted and scar area was measured. The time required (in days) for complete epithelisation was noted.

Resutured incision wounds:

After clipping the hair from the back, two full thickness, parallel, paravertebral incisions of 6cm long, 1 cm lateral to vertebral column were made as described earlier by Ehrlich and Hunt[7].Wounds, after complete haemostasis were closed with 5 interrupted sutures placed 1 cm apart using 4-zero silk thread. The sutures were removed on 8^{th} post-wound day and the breaking strength of the wound was estimated on 10^{th} day of wounding by continuous, constant water flow technique as described by Lee[8].Gaping of wound was taken as end point to note the breaking strength. Minimum three readings were recorded in each wound.

Dead space wounds:

These wounds were caused by implanting sterile cotton pellets of 10 mg and 2.5 cm long sterile grass piths subcutaneously either in axilla or in groin at random by the technique of D'Arcy et al as described by turner[9].Each animal received two cotton pellets and two grass piths. On 10^{th} post wounding day, the cotton pellets were dissected out and dried overnight to take their dry weight next day. Granulomatous tube surrounding pith was dissected out and slit open longitudinally. After trimming each end the rectangular piece of 2 cm long granulation tissue was used to estimate the breaking strength. One end of the tissue was fixed with the help of blunt forceps, while the other end was connected to water container through another blunt forceps via a pulley. Partial or complete tear in granulation tissue, at any point in between the points of forceps application was taken as an end point to note the breaking strength. Subsequently, the pieces of granulation tissue were kept in 10% formalin solution for further histological studies. The dry weight of cotton pellet granuloma was expressed as mg% of body weight as suggested earlier[10] to account for the influence of nutrition on granuloma formation.

Blood counts were carried out in all the animals before pentazocine administration and on 5^{th} day of wounding. The animals inflicted with incision and dead space wound were sacrificed on 10^{th} day after estimating breaking strength and removing granulomas to study changes in adrenals and thymus. The adrenals and thymus were removed and the weight was recorded.

All the animals were caged seperately after wounding to monitor their daily food intake and body weight changes. None of the animals received systemic or local antiinfective agents during the study and infected animals were excluded from the study.

All the procedures were performed in accordance with the CPCSEA guidelines and the study was approved by IAEC.

Statistical Analysis: Data were expressed as Mean ± SEM and analysed by student 't' test and $p \leq 0.05$ was considered as significant.

Results

Excision wound closure rates and mean raw area in pentazocine animals did not differ significantly ($p>0.05$) at any day of observation, from that of control animals (Table I).On complete epithelisation the scar shape in pentazocine animals was almost alike that of controls with cicatrisation. The mean scar area in pentazocine group did not significantly differ from the corresponding control values (Table I). There was no significant difference in number of days required for complete epithelisation (Table I).

The breaking strength was significantly decreased in pentazocine treated animals both in resutured incision wound as well as granulation tissue as compared to saline treated controls (Table II). Pentazocine also significantly suppressed granuloma formation (Table II).

Haematoxylin and eosin stain sections of granulation tissue when observed under microscope (Fig I), the population of fibroblasts and collagen deposition were markedly decreased in pentazocine group in contrast to control animals.

Haemoglobin contents and WBC counts in pentazocine animals on 5th day from starting drug treatment did neither differ from their own pre-drug values nor from corresponding value of control animals.

The mean weight of adrenals and thymus in pentazocine animals did not differ significantly from the corresponding weight in control animals (Table III).There was no significant ($p>0.05$) difference in food intake and the bodyweight changes between treated and control animals (values not shown).

Table I. Effect of pentazocine on excision wound healing.

Groups (n=6)	**Drugs and Dose mg/kg**	**Raw wound area(mm^2)**			**Time for complete closure (days)**	**Scar area (mm^2)**
		Day-4	**Day-12**	**Day-16**		
		Mean ± SEM				
1	Control	429.0±25.90	39.0±4.90	8.3±1.50	19.2±0.30	56.5±11.12
2	Pentazocine 2.70	453.0±2.46	44.2±2.60	13.3±6.70	19.5±0.70	60.7±11.19

Student ‘t’ test. No significant change in any of the parameters, in treated group.

Table II. Effect of pentazocine on incision wound.

Groups (n=6)	Drugs and Dose mg/kg	Breaking strength (G)		Granuloma dry weight (mg% b.w)
		Resutured incision wounds	Granulation tissue	
		Mean ± SEM		
1	Control	280.00±13.60	358.00±50.00	53.0±3.60
2	Pentazocine 2.70	224.67±9.20*	231.00±10.00*	33.0±3.70*

Student 't' test, $p<0.05$*

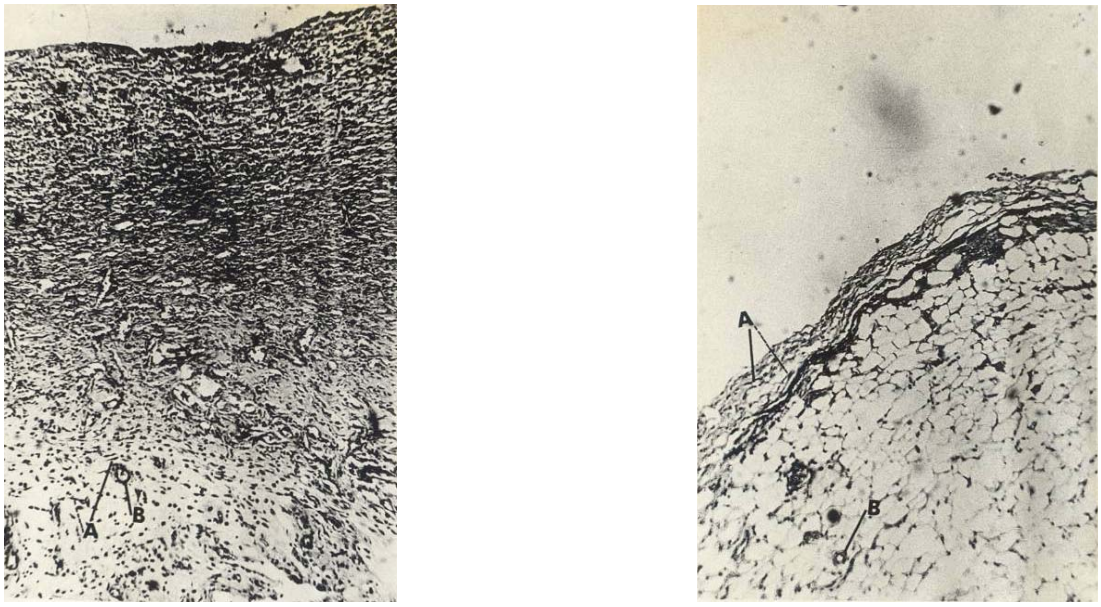
Table III. Effect of pentazocine on thymus and adrenals weight.

Groups (n=6)	Drugs and Dose mg/kg	Weight mg/100 g body weight Mean±SEM	
		Thymus	Adrenals
1	Control	43.49±4.30	21.75±3.52
2	Pentazocine2.70	46.65±2.40	20.95±3.17

Student't' test . No significant change in weights of thymus and adrenals in pentazocine treated animals.

Figure I . Microphotographs of granulation tissues stained with H&E (100X)

i) Control (Saline) ii) Pentazocine (2.70 mg/kg)



Note: A-Fibroblasts, **B**-Capillaries.

Pentazocine treatment markedly (ii) decreased the thickness of granulation tissue, fibroblast number and collagen content as compared to control (i).

Discussion

Pentazocine significantly($p<0.05$) suppressed healing of incision wounds and dead space wounds as observed by decreased breaking strength of 10 day old incision wounds and that of granulation tissue. It also suppressed granuloma formation without any significant effect on healing of excision wound. Similar studies about pentazocine have not been documented in the literature. However like pentazocine calcitriol, a reported cytotoxic agent when tested on wound healing significantly retarded healing in Wistar rats[11].

The complex process of healing consists of epithelisation, granulation, collagen deposition, contraction etc. The contribution of individual process to healing, depends upon the type of wound e.g. epithelisation assists more than collagenisation for healing of excision wounds and collagenisation is important in healing of incision wounds. These various processes of healing are independent of each other and one of them might be selectively suppressed without affecting the other viz., Vit C deficiency affects collagenisation without affecting wound contraction[12].Therefore, any drug that hinders one of these processes of healing might obviously retard, the healing of one type wound without affecting other wound model.

The incision wounds, mainly heal by collagenisation, while the dead space wounds by granulations. Estimations of breaking strength of incision wound in order to assess collagenisation is well known.Quantification of foreign body granuloma tissue, by noting its dry weight has been used to assess wound healing[13,14].Since, collagen deposition is a function of fibroblast, a component of granulation tissue, any agents that affects granulation tissue could also be expected to affect to collagenisation. In the present study, pentazocine has affected the healing of both incision as well as dead space wound, probably, by suppressing fibroblast proliferation in granulation tissue and possibly interfering with collagen deposition. Histological study of granulation tissue also support healing suppressant effect on pentazocine, as indicated by paucity of fibroblasts and collagen treated group. Histological study of granulation tissue has been employed in wound healing studies earlier[15].The suppressant effect on pentazocine on granulation tissue formation could be due to its reported antiproliferative action[4] and not through endogenous mechanisms such as corticosteroids release, since the weight of thymus and adrenals are not affected by pentazocine. This is further supported by the fact that healing of excision wound is not retarded by pentazocine.

It is difficult to explain why antiproliferative action of pentazocine does not affect the healing of excision wound. In the present study pentazocine selectively suppressed granulation (sub epithelial) tissue rather than epithelium. Such disassociation in epithelial and subepithelial healing has been reported with topically applied hydrocortisone to excision wounds[16].Since pentazocine does not alter the adrenal weight the alternative explanation could be, small concentration of drug reaching epithelium to suppress its growth. However further study is essential to explore the mechanism.

The present study not only shows that pentazocine adversely affect wound healing, but also points out indirectly that use of single wound model in such studies might not reveal the true nature of the drug and therefore, would give inadequate information leading to wrong conclusions. If the present results were to be extrapolated to clinical situation, pentazocine as a post operative analgesic is likely to increase wound complications. However, the clinical studies in this regard are worthwhile, since pentazocine is used commonly as post operative analgesic.

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