METRONIDAZOLE ON BURN HEALING

INTRODUCTION

One of the complications of the burn injuries is life-threatening infection, nearly 15% of which are caused by anaerobic bacteria. Metronidazole that controls anaerobic infections is administered orally, intravenously or topically depending on the severity of the infections in burn-wound patients. Whether metronidazole, topical/systemic, influences healing of burn wounds is not precisely known. Rice, reported that topical metronidazole improved wound appearance of infected pressure sores, leg ulcers etc. This could be due to either bactericidal or direct pro-healing property of the drug. Prasad and Rao observed that oral metronidazole increased wound contraction and epithelization in non-infected skin excision wounds suggesting that it has direct beneficial effects on healing process. However, the reported cytotoxic effect of metronidazole in tissue cultures questions the suggested pro-healing effects of the drug. One can surmise that the available data on the effects of metronidazole on wound healing are conflicting, at best, equivocal. There are no studies focusing on the effect of metronidazole on healing particularly on burn wound. The focus of the present study is precisely that.

Recently, a topical preparation of metronidazole namely metronidazole gel USP is being used to treat anaerobic (B. fragilis) burn wound infections. Topical chemotherapeutic preparations meant for burns should not inhibit the reepithelization and cause injury to viable cells. Not only the chemotherapeutic component but also the base used should not inhibit healing. There is a misplaced general impression that the bases of topical preparations are inert and do not materially affect the healing. This has been shown to be a naïve impression. Jamader et al found that some ointment bases affected the healing. Goel and Ahuja, who found that some synthetic gel materials

AN APPRAISAL OF THE HEALING PROFILES OF ORAL AND EXTERNAL (GEL) METRONIDAZOLE ON PARTIAL THICKNESS BURN WOUNDS

Objective: To assess the influence of oral and topical (gel) metronidazole on partial thickness thermal burn wound healing.

Methods: Partial thickness burn wounds were produced on dorsum of rats by pouring hot molten wax at 80°C. Oral and two topical gel (MGEL-1 and MGEL-2) preparations of metronidazole or their corresponding vehicles were administered to different groups of wound bearing animals. The effects of the treatments were compared by periodically observing wound contraction and epithelization and histological features as indicative of the progress of healing.

Results: Healing of burn wounds was significantly promoted by oral metronidazole (p<0.01) and the gel base of MGEL-2. In contrast, the MGEL-1 significantly (p<0.05) depressed the epithelization process while its base was without effect, indicating that topical metronidazole retards healing. The effect of MGEL-2 appeared to be an algebraic sum of pro- and anti-healing effects of the base and drug respectively more in favour of the base (p<0.05).

Conclusion: Metronidazole orally promotes but topically depresses healing of burn wounds. The latter effect can be reversed if the base has pro-healing property.

SUMMARY

KEY WORDS

Burn wounds  healing  metronidazole  wound contraction  epithelization
used as dressings have accelerated healing further corroborate this finding. Hence the present study extends its scope to investigate the effect of gel-base/s used in two of the marketed metronidazole topical gel preparations namely MGEL-1 and MGEL-2.

**MATERIALS AND METHODS**

**Drugs:** Metronidazole oral: Pure metronidazole bulk powder (courtesy M/s QUES Pharmaceuticals, Ongole, A.P) was suspended in 0.5% carboxy methylcellulose (CMC) and administered to rats p.o.

Metronidazole external: Two marketed metronidazole gel USP preparations (containing metronidazole in the strength of 10 mg/g of the gel) and their water soluble bases namely Metrogyrl® gel and its base (courtesy M/s Unique Pharmaceutical Labs, Bombay), & Largyl® gel and its base (courtesy M/s Lark Labs, New Delhi) were employed in the study.

**Animals:** Healthy male Wistar rats weighing between 180-200 g were used in the studies. They were individually housed and maintained on normal diet and water ad libitum.

**Burn wound model:** Partial thickness burn wounds were inflicted, on overnight-starved animals under pentobarbitone (30 mg/kg, i.p.) anaesthesia, by pouring hot molten wax at 80°C into a metal cylinder of 300 mm² circular openings placed on the shaven back of the animal[10]. Immediately after the injury and on subsequent days Ringer lactate (1 ml/kg) was administered i.p., daily for resuscitation. Apart from the drugs under investigation no local/systemic chemotherapeutic cover was provided to animals. Animals showing the signs of infection were excluded from the study and replaced with fresh animals.

**Experimental protocol:** Animals bearing the partial thickness burn wounds were distributed into various groups each containing 10 animals to study the effect of oral and external metronidazole on burn healing.

**Oral metronidazole:** Two groups of animals received either vehicle (0.5% CMC) or metronidazole (180 mg/kg) daily in the volume of 2.5 ml/kg, p.o., till the healing completed. The dose was arrived at by computing[11] the highest clinically recommended dose[12] of metronidazole to rat.

**External metronidazole:** Five groups of animals were used in this study. The first group (control) received no topical application. The other four were given thin layers of topical applications on their wounds either with metronidazole gel preparation-1 (MGEL-1), its base (Base of MGEL-1), Gel preparation-2 (MGEL-2), or the base of the Gel preparation-2 (Base of MGEL-2) in the quantity of 100 mg OD, till healing completed.

**Assessment of burn healing:** Animals were inspected daily and the healing was assessed based on physical parameters namely, wound contraction and epithelization as well as histologically.

**Wound contraction:** Studied by tracing the raw wound area on a transparent polythene paper on day 2, 6, 10, and 14 post wounding days. Later the area assessed using a graph paper. The wound contraction was measured as the percentage decrease of original wound size 300 mm² for each animal of a group.

**Reepithelization:** Falling of eschar leaving no raw wound area was considered as end point of complete reepithelization and the days required for this was taken as period of epithelization.

**Histopathology:** On day 10 some of the animals under each group were sacrificed and the wounds excised together with the surrounding skin. They were fixed in 10% neutral buffered formalin. Histological evaluation was performed on haematoxylin and eosin (HE) stained 5-6 µ thin paraffin sections of wound bed material.

**Statistical Analysis:** Results are reported as mean ± SEM. The data was analyzed by unpaired Student’s ‘t’ test in case of oral metronidazole study and one way ANOVA followed by Studentized Range Procedure as described by Bolton[13] in case of topical metronidazole study.

**RESULTS**

Oral metronidazole: In control animals wound contraction was to the extent of 33%, 56%, 66%, and 87% by day 2, 6, 10, and 14 respectively. These animals took 14.25 ± 0.45 days for reepithelization. Metronidazole administered orally shortened the period of epithelization significantly (p<0.01) by 3 days. Besides, it also promoted the wound contraction throughout (Table 1).
Table 1. Effect of oral metronidazole on epithelization and wound contraction of partial thickness burn wounds.

<table>
<thead>
<tr>
<th>Drug (n)</th>
<th>Dose (mg/kg)</th>
<th>Period of epithelization (days)</th>
<th>Wound contraction on days#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Control (10)</td>
<td>2.5ml</td>
<td>14.25 ± 0.45</td>
<td>33 ± 2.5</td>
</tr>
<tr>
<td>(0.5% CMC)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Metronidazole (10)</td>
<td>180</td>
<td>11.12 ± 0.87*</td>
<td>40 ± 1.7*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM n = no of animals; * = p < 0.01 Vs control.
#Reduction in wound area as % of original wound size (300 mm²).

Table 2. Effects of topical metronidazole (gel) preparations and their bases on epithelization and contraction of partial thickness burn wounds.

<table>
<thead>
<tr>
<th>Drug (n)</th>
<th>Period of epithelization (days)</th>
<th>Wound contraction on days#</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Control (10)</td>
<td>15.55 ± 0.53</td>
<td>32 ± 2.3</td>
</tr>
<tr>
<td>Base of MGEL-1 (10)</td>
<td>15.77 ± 0.59</td>
<td>32 ± 1.35</td>
</tr>
<tr>
<td>MGEL-1 (10)</td>
<td>20.12 ± 0.83*</td>
<td>35 ± 3.5</td>
</tr>
<tr>
<td>Base of MGEL-2 (10)</td>
<td>11.11 ± 0.87*</td>
<td>38 ± 1.6</td>
</tr>
<tr>
<td>MGEL-2 (10)</td>
<td>17.02 ± 0.66*</td>
<td>38 ± 3.3</td>
</tr>
<tr>
<td>Allowance value*</td>
<td>2.05</td>
<td>6.5</td>
</tr>
</tbody>
</table>

Values are mean ± SEM n = no of animals per group; *p < 0.05 Vs control; b p < 0.05 Vs respective bases. c p < 0.05 Vs MGEL-1. * = any two means differing by the said allowance value are statistically different.
#Reduction in wound area as % of original wound size (300 mm²).

Histological examination performed on the ten-day old wounds showed a steady and progressive wound healing in control animals. The dermis proliferated almost to reach normal level. Eschar was getting separated off leaving space for epidermis to grow and complete reepithelization. Moderate amount of collagen and numerous inflammatory cells could be seen in corium (Figure 1). However, wounds in oral metronidazole treated animals showed signs of advanced healing such as complete restoration of epidermis, well organized high amount of collagen bundles in dermis, and absence of inflammatory cells in fully grown dermis (Figure 2).

Topical metronidazole: While the base of MGEL-1 did not affect the healing, MGEL-1 suppressed the epithelization process significantly (p<0.05) and prolonged the period of epithelization by four days (Table 2). The healing depressant effect of external metronidazole was also observed in the histology sections of 10-day old wounds. Lots of infiltrated inflammatory cells could be seen in the deeper portions of the dermis of the wounds treated with MGEL-1. Importantly, the growing epidermis over the layers of dermis could not be seen unlike that could be seen in control wounds (Figure 3). Although, these wounds are 10-day old, the histological feature suggests that these wounds be still in the early stages of healing. It is evident from these findings that the topical application of metronidazole depresses the burn healing. The findings, however, with the MGEL-2 and its base were contrary to what were observed with MGEL-1 and its base. The base of MGEL-2 itself significantly (p<0.05) promoted the epithelization and contraction while the MGEL-2 did not significantly depress the reepithelization (Table 2). The healing promoting
Figure 1. Photomicrograph of 10-day old burn wound of control group (H & E X 100).

Dermis proliferated to reach normal level. Eschar getting separated just above the dermis. Moderate collagen and numerous inflammatory cells could be seen in corium.

Figure 2. Photomicrograph of 10-day old burn wound of oral metronidazole group (H & E X 100).

Signs of advanced healing - complete restoration of epidermis, presence of rich and well organized collagen and absence of inflammatory cells in fully grown dermis.

Figure 3. Photomicrograph of 10-day old burn wound of topical MGEL-1 group (H & E X 100).

Poorly healed wound - absence of layers of growing epidermis, presence of lots of inflammatory cells in deeper portion of dermis.

Figure 4. Photomicrograph of 10-day old burn wound of topical application of the base of MGEL-2.

Moderately healed wound - fully grown epidermis either into or under the eschar, presence of well organized collagen bundles in dermis.
action of the base of MGEL-2 was confirmed histologically as well. Epidermis had attained its continuity by growing either into or under the eschar, notwithstanding that the latter was still intact. The cells of the dermis were almost similar to that of normal region. The collagen bundles were organized in the well-marked neo-vascularized matrix (Figure 4). Perusal of the data of (Table 2) reveals that MGEL-2 has significantly (p<0.05) lesser healing-depressant effect than MGEL-1. Thus, MGEL-2 is devoid of any depressant effect on burn healing.

DISCUSSION

The results of the present study show that oral metronidazole accelerates healing while external metronidazole depresses it. On the other hand one of the bases of the gels promotes healing while the other one is neutral to healing.

The pro-healing effect of oral metronidazole in burn wounds corroborates with the findings of Prasad and Rao who reported that metronidazole would promote contraction and epithelization of excision wounds. However, this is contrary to what was observed by Borden in fascial wounds. While the systemic metronidazole did not materially alter contraction of skin wounds it did depress the breaking strength of fascial wounds.

The report that metronidazole is cytotoxic to the mammalian cells growing in vitro supports the healing depressive effect of topical metronidazole observed in the present study. However, topical metronidazole improves the appearance of wound infected with bacteroids. The elimination of infection by metronidazole eventually leading to promotion of healing could not be ruled out in this case.

The present study reveals that the bases of the topical formulations could interfere with the healing process. Both MGEL-1 and 2 contain water soluble gel bases. While the base of MGEL-1 has not affected the healing, the base of MGEL-2 has shown pro-healing effect. The composition of these two bases is different. The base of MGEL-1 comprises carbomer, methyl paraben, propyl paraben, disodium edetate, propylene glycol and water for injection. On the other hand the base of MGEL-2 consists of carbopol, isopropyl alcohol, diethanolamine and allantoin. Some of the constituents of the base of the MGEL-2 could have played a role in promoting the healing. For instance allantoin. Allantoin has been used in psoriasis and other skin diseases usually in the form of creams and lotions. Interestingly, allantoin is claimed to stimulate cell proliferation, tissue formation and hasten wound healing. The bases of topical applications interfering with healing is not surprising, as different ointment bases have been shown to affect healing differently. Moreover, some of the dressing gel materials have been reported to accelerate burn healing.

While MGEL-1 significantly (p<0.05) depressed the healing, the MGEL-2 has not done so. When the effects are compared with their respective bases employed it is evident that metronidazole at 1mg (in the form of gel) significantly depressed wound healing. The depressant effect of metronidazole appears to be so powerful as to over-shadow the pro-healing effect of the base of MGEL-2. The period of epithelization of 11 ± 0.87 days has been increased to 17.02 ± 0.66 days. Between MGEL-1 and 2 the latter possesses significantly lesser depressant effect than the former. It is therefore clear that the pro-healing effect of the base of MGEL-2 has reversed the healing-depressant effect of topical metronidazole.

It is intriguing that oral metronidazole promoted while, topical metronidazole depressed the healing. Although the present study is not aimed at exploring the mechanisms for the pro- and anti-healing effects of metronidazole, the latter mentioned effect could be attributed to the known direct cytotoxicity of metronidazole. In a cell culture study at a concentration of 29 mM (4.5 mg/ml) metronidazole was found to depress the viability of Chinese hamster ovary cells and to inhibit DNA synthesis in mouse L-929 cells. Thus, at a dose of 1 mg, topical metronidazole could also be expected to home in, in high amounts, on the reparative cells causing them direct cytotoxicity. On the other hand, it is difficult to explain how oral metronidazole has promoted healing despite the fact it is cytotoxic. Perhaps, only a small fraction (less than 0.1mg) of the orally administered drug would be reaching the wound area and in such small concentration level it might not be cytotoxic. The assumption that only 0.1mg of orally administered metronidazole could have reached the
wound is based on the following parameters. Average weight of a rat -200 g, BSA -0.0317 m$^2$, Blood volume -14 ml, oral bioavailability of metronidazole - >95%, oral dose of metronidazole -36 mg/ 200 g rat, and blood supply to the skin - 1/3 of the blood volume. However, systemic metronidazole could still be assumed to have scavenged the free radicals and prevented the generation of lipid peroxides that are known to be present during burn stress$^{18}$. A reduction in lipid peroxides of burns may reduce the further loss of tissue in burned area and may thus promote healing. If this were to be true one would expect similar findings with topical metronidazole. We opine that the direct cytotoxicity of topical metronidazole, in such a high concentration, might have a more pronounced retarding effect on the healing as compared to its indirect effect as an antioxidant. However, this needs a detailed appraisal.

Topical anti-infective formulations are routinely employed in burn wounds. The bases are assumed to be inert. However, in the light of the present study bases cannot be assumed to be innocuous. They could either facilitate or retard healing. Therefore, it is imperative that a thorough evaluation of bases used in topical formulation be done prior to the incorporation of medicament.

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REFERENCES