Development and evaluation of transdermal formulations containing metronidazole and norfloxacin for the treatment of burn wound

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In an attempt for better treatment of partial thickness burn wounds topical ointments containing metronidazole and norfloxacin in different bases were prepared and in vitro release was conducted in phosphate buffer pH 6. It was found that, diffusion of the metronidazole and norfloxacin from the lanolin petrolatum base with 0.25% w/w dimethyl sulfoxide was maximum through hairless rat abdominal skin. Antimicrobial activity of different prepared formulations was found to be more effective both against aerobic and anaerobic bacteria than marketed formulation (1% silver sulfadiazine cream USP). Formulations were significantly effective as compared to that of marketed formulation in wound contraction of the partial thickness burn wound. Histopathological reports supported effectiveness of formulations. It was found that 1% metronidazole and 1% norfloxacin ointments are suitable for treating the partial thickness burn wound.

Infection is a major complication of burn injury and is responsible for 50% to 75% of hospital deaths. Moist thermally coagulated burn wound, with its constantly replenished supply of diffusing serum nutrients and its warm surface temperature, provides an environment suitable for rapid microbial growth. As local microbial growth increases, potential for invasion of subjacent viable tissue and penetration into circulation increases.

Microorganisms that cause burn wound infections have changed over years related to changes made in the treatment of burn patients. Ramakrishnan et al. and Wang et al. reported separately that anaerobic bacteria is the causative organisms of infections in around 15% of burn infected patients. Ramakrishnan et al. used metronidazole either orally or intravenously depending on severity, to anaerobically infected burn wound in patients.

Rice, reported that metronidazole caused an improvement in wound appearance when applied externally to infected pressure sores, leg ulcers, etc. This could be due to either bactericidal or direct pro healing property of the drug.

Hou-zI reported that silver norfloxacin proved valuable in treatment of burn wound infection caused by invading organisms, particularly by silver sulfadiazine resistance strain of Pseudomonas.

Usually the general impression on topical medicines is that the base of the preparations are inert and do not materially effect the healing. But recent findings by Jamadar et al. showed that some of ointment bases affect healing. Hence, it is worthwhile to check if the base itself has got any influence on burn healing.

Objectives of the present studies was to prepare Metronidazole and Norfloxacin ointments using different bases. Ointments were evaluated for drug release using rat skin, antimicrobial activity against aerobic and anaerobic microorganisms, wound healing (contraction time) and histopathological changes in rats after induction of artificial burn.

Materials and Methods

Materials—Norfloxacin and metronidazole were a kind gift from Cipla Ltd., (Mumbai, India) and Karnataka Antibiotic and Pharmaceutical Ltd. (Bangalore, India) respectively. 1% silver sulfadiazine cream USP was procured from Kasturba Hospital manufacturing unit (Manipal, India).

Composition of the ointments—Formulation I (FI) containing 1% norfloxacin and 1% metronidazole and 0.25% DMSO in water soluble polyethylene glycol ointment base which contains polyethylene glycol 6000 (40.0g), polyethylene glycol 400 (60.0g). formulation II (FII) containing 1% norfloxacin and 1% metronidazole and 0.25% DMSO in lanolin
petrolatum ointment base which contains petrolatum (50.0g), wool fat (30.0g), hard paraffin (4.0g), liquid paraffin (16.0g). Formulation III (FIII) containing 1% norfloxacin and 1% metronidazole and 0.25% DMSO in emulsion ointment base (w/o) type which contains white soft paraffin (20.0g), white bees wax (20.0g), wool fat (10.0g), liquid paraffin (30.0g), borax (1.0g), distilled water up to 100.0g.

In vitro drug release using hairless rat abdomen skin—Healthy male Wistar albino rats weighing 150-200g were used. Freshly excised rat abdominal skin was taken and cut into the required size and mounted on a specially designed cylindrical diffusion cell of glass (inner diam. 3.3 cm) such that stratum corneum was facing the donor compartment. Two g of ointment was placed inside this and spread uniformly such that it occupies the inner circumference of the diffusion cell. The skin was firmly fixed to the cell with the help of rubber bands. The release was carried out in 100 ml of receptor medium (phosphate buffer (pH 6) for 8 hr and the medium was stirred at a constant speed (100 rpm) with the help of magnetic stirrer to ensure uniform mixing of contents. At predetermined time intervals aliquot was withdrawn and replaced by same amount of the medium. The absorbance of the sample was measured at wavelengths 272 and 320 nm for norfloxacin and Metronidazole respectively. The concentrations were calculated by simultaneous equations method 7,8.

Estimation of drugs in base—After the in vitro release the ointments remaining on the skin was extracted with 50 ml of the glacial acetic acid. The absorbances of the sample were measured against blank (bases treated in the same manner without drugs) at wavelengths 280 and 305 nm for norfloxacin and metronidazole respectively. The amount of the drug remaining was calculated by simultaneous equations 8.

Estimation of drugs in skin—After in vitro release the skin was washed using phosphate buffer pH 6 and cut into 3x3 cm size. The drugs was extracted using glacial acetic acid after homogenization. The blank was also prepared by taking fresh skin of similar dimension and processed in the same manner as above. The drugs extracted was analysed at wave length 280 and 305 nm for norfloxacin and metronidazole respectively 8.

Microbiological studies—Psuedomonas aeruginosa in Nutrient agar medium was used for aerobic study while Bacteroides fragilis, Porphyromonas gingivalis in blood agar medium were used for the anaerobic study.

Nutrient agar medium (100 ml) was inoculated with Ps. Aeruginosa (1%v/v) culture. 10 mg of the formulations (F1, FII, and FIII) and the marketed preparation (MF-1% Silver sulfadiazine cream USP) were weighed (10mg containing 100µg of the drugs) on a small circular Whatmann filter paper disc which was kept inverted on the nutrient agar medium. Aseptic and sterile conditions were followed during the studies. The medium containing the formulations was incubated at a temperature of 37±0.2°C for 24 hr. The inhibition zone diameters were measured with the help of zone reader. The blood agar medium was inoculated with the anaerobic cultures using swabs and the remaining procedure was followed same as described under aerobic studies. The plates are then incubated under H2/CO2 in a anaerobe jar at 37°C. Inhibition zones were measured after 48 hr of incubation 9.

Animals—Healthy male Wistar albino rats weighing between 150-180g were used. They were individually housed and maintained on normal diet and water. Animals were randomly distributed into various groups each containing 10 animals.

Inflicting wax burn wound—Burn wounds were inflicted on overnight starved animals under pentobarbitone sodium (6 mg/100 g, ip) anesthesia. Apart from the drugs under investigation, no local/systemic chemotherapeutic covers were provided to animals. On zero day, under anesthesia, the dorsum of each rat was shaved. A 2x2 cm metal cylinder was placed on the shaven back of the animals. To this was poured melted wax at 80°C and the wax was allowed to solidify into metal cylinder. Eight minutes after this (during the wax solidified completely), the metal cylinder containing solid wax adhering to the layers of skin was gently removed to inflict a distinctly demarked burn wound 10.

Assessment of burn wound healing—Formulations, and market preparation were applied to the different groups of animals (500 mg) on every alternate day from day 2. Animals were observed daily and the healing was assessed by measuring the wound contraction (tracing the raw wound area on a transparent polythene paper which were retracted on a graph paper to assess the area) up to 16 th day post wounding. The wound contraction was calculated as percentage of original wound size for each animal of a group 10.
Histopathological studies—On 0, 2nd, and 15th day, one of the animals under each group was sacrificed and the wound was excised together with the surrounding skin. They were fixed in formalin and embedded in paraffin. Histological evaluation was performed on the haematoxylin and eosin-stained paraffin section.

Results
The in vitro release profile of norfloxacin and metronidazole from different formulations is shown in Fig. 1A&B. The release of the drugs from the formulation II (1% norfloxacin and 1% metronidazole and 0.25% DMSO in lanolin petrolatum base) was higher compared to the other formulations. In case of the formulation III (1% norfloxacin and 1% metronidazole and 0.25% of DMSO in w/o emulsion base) the release of metronidazole was found to be higher than Norfloxacin contrary to other formulations.

Formulation I (1% norfloxacin and 1% metronidazole and 0.25% of DMSO in PEG base) had highest antimicrobial effect against both aerobic (Ps. Aeruginosa) and anaerobic (B. Fragilis, P. Gingivilis) bacteria as compared to that of other
Fig 4—Histological section of an experimental partial thickness burn, on 15th day After treatment: A-Marketed formulation-Initiation of epithelialization (→); B-With formulation I-Regeneration of epidermal layer (→); C-With formulation II-Keratinisation (→); D-With formulation III-Healthy broad epidermal layer (→)
formulations. Marketed formulation (1% silver sulfadiazine cream USP) did not show any antibacterial activity against anaerobic bacteria (Fig. 2). Microbiological studies were also conducted with norfloxacin and metronidazole individually where norfloxacin had a distinct zone of inhibition against aerobic bacteria and metronidazole showed no antimicrobial activity. Burn induction resulted in distinct demarked wound on day 0. At the end of the day 1 eschar could be seen which becomes harder and prominent on day 2. Wound contraction after application of different formulations progressed significantly \( (P < 0.05) \) as compared to marketed formulation (Fig. 3).

Histopathological examinations of the wounds showed the following features. On day 15, healing of the wounds was progressing steadily with the applications of the different formulations. Treatment with marketed formulation, shows initiation of epithelialization (Fig. 4A). Treatment with formulation I, shows re-epithelialization (Fig. 4B). With formulation II, adnexial glands, thin epidermal layer and keratin production is seen (Fig. 4C). Thus the healthy recovery exhibited by formulation II is in accordance with its in vitro release through the skin. With formulation III, healthy broad epidermal layer is seen (Fig. 4D).

Discussion

In case of formulation II (containing lanolin petrolatum base) release of the drugs was higher as compared to other formulations. Petrolatum, being a hydrocarbon base is occlusive and increases the skin hydration by decreasing the loss of surface water whereas lanolin being an absorption base, has the ability to absorb more water thus increasing the percutaneous absorption by hydration. The synergistic effects of these components may be responsible for increased drug release. In case of formulation III (containing w/o type emulsion base), the release of Metronidazole was found to be higher than Norfloxacin contrary to other formulations. This may be attributed to the difference in hydrophobicity of the drugs. Norfloxacin being more hydrophobic than metronidazole gets partitioned in the oil phase resulting in less availability of norfloxacin for the release. These are further supported by the results of the residual analysis (Table 1), where the amount of Norfloxacin remaining in the base after in vitro release was more than that of Metronidazole from formulation III and vice versa in case of formulation II.

Analysis of wound contraction results showed that prepared formulations were more effective than marketed formulation. Histopathological studies revealed that prepared formulations have shown better healing property by promoting keratinization and re-epithelialization. Histopathological studies is a better indication than wound contraction which is a superficial and visual index to measure wound healing. Therefore prepared formulations, forming an occlusive barrier enhanced re-epithelialization by preventing wound dessication and appeared to be better formulation for burn wound healing. These findings are in accordance with the earlier report of Kanon and Garrett.

References