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STABILITY, IN-VITRO AND IN-VIVO RELEASE STUDIES FROM METRONIDAZOLE OINTMENTS

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ABSTRACT

The influence of ointment formulation on the stability, the in-vitro release and the in-vivo absorption through the skin of rabbits was investigated. The choice of the selected ointments has no influence on the drug stability with the exception of an acidified emulsion base. A good correlation between in-vitro release and in-vivo absorption was found revealing that metronidazole was quickly released and effectively absorbed from a polyethylene glycol base.

INTRODUCTION

Metronidazole has been used for over 20 years in the treatment of various anaerobic infections (Brogden et al., 1978).

Recently patient with rosaceae are treated with either metronidazole ointments or with metronidazole tablets (Nielsen, 1983).

The present communication is concerned with the stability of metronidazole in several ointments, the in-vitro release rate of metronidazole from ointments and the percutaneous resorption in rabbits.

EXPERIMENTAL

Chemicals

The following chemicals and reagents were used to prepare the ointments and to performe the analysis : metronidazole and tinidazole (Sigma Chemical Company, St. Louis, USA), white soft paraffin, liquid paraffin, polyethylene glycol 4000 and 400, sodium lauryl sulphate, methylparahydroxybenzoate, propylparahydroxybenzoate and Carbopol 940® (Pharmachemic, Antwerpen, Belgium), cetyl alcohol, white wax and propyleneglycol (Vel-Bios, Leuven, Belgium), Cutina G.M.S.®, Emulgin B_2, Myritol 318® (Henkel & Cie, GmbH, Düsseldorf, West-Germany). Sodium hydroxide, lactic acid, zinc sulphate and ethanol, all p.a. grade, (U.C.B., Leuven, Belgium). Disodium phosphate p.a. and monopotassium phosphate p.a. (Merck, Darmstadt, West-Germany). Citric acid p.a. and methanol of chromatographic grade (Carlo Erba, Milan Italy). Idroramnosan® (hydroxyethylcellulose 4500 mPa.s.), (Etabl. Arion, Brussels, Belgium).

Preparation and storage of ointments

Five ointment bases were investigated in this study and the composition of these bases are presented in Table 1. The metronidazole was incorporated at a level of 0.5 %, 1 % or 2.5 % (W/W). All suspension ointments showed drug particles between 10 µm and 100 µm. Besides the ointments described in Table 1, an emulsion base (type B_2) was prepared with 1 % lactic acid as described

TABLE 1.
Composition and type of ointment bases used.

Ointment	% drug concentration	Type of base	Composition	
A	0.5[x] 1[x] 2.5[x]	Water soluble	Polyethylene glycol 4000 Polyethylene glycol 400	20.0 g 80.0 g
B_1	0.5[x] 1[xx] 2.5[xx]	O/W emulsion	Cetyl alcohol White wax Propylene glycol Sodium lauryl sulphate Buffer solution	15.0 g 1.0 g 10.0 g 2.0 g 72.0 g
B_2	0.5[x] 1[xx] 2.5[xx]		Cutina GMS (glycerylmonostearaat) Emulgin B_2 (oxyethylated cetostearyl-alcohol) Liquid paraffin Myritol 318 (Caprilic triglyceride) Buffer solution	13.0 g 3.0 g 5.0 g 5.0 g 74.0 g
C	0.5[xx] 1[xx] 2.5[xx]	Oleaginous	Paraffin wax Liquid paraffin	80.0 g 20.0 g
D_1	0.5[x] 1[xx] 2.5[xx]	Hydrogel	Carbopol 940 Sodium hydroxide solution (10 %; W/V) Water	1.0 g 8.3 g 90.7 g
D_2	0.5[x] 1[xx] 2.5[xx]		Hydroxyethylcellulose (4500 mPa.s.) Water	0.5 g 99.5 g

Metronidazole is incorporated in the base by dissolution[(x)] or by levigation[(xx)]

by Nielsen (1983). The buffer solution used for the preparation of the emulsion bases consists of 0.112 g/100 ml citric acid and 0.039 g/100 ml disodium phosphate and was selected in order to keep an optimal pH for the metronidazole stability (Kraus and Vermeij, 1981). All ointments were stored in aluminium tubes at both 40 °C and 4 °C.

In-vitro release from ointments

Stainless steel circular receptors (45 mm diameter; 5 mm depht) were filled with ± two grams ointments. The excess was removed and the receptor was covered with a hydrophilic cellulose acetate membrane (0.45 µm pore size, Sartorius GmbH, Göttingen, W-Germany). The cells were immersed in a 400 ml beaker containing 200 ml distilled water. The beakers were then placed in a constant temperature water bath at 37 ± 1 °C and the diffusion was allowed to proceed. The amount of metronidazole diffused was assayed continuously using a spectrofotometer equipped with flow cells (Zeiss PM 6, West-Germany) and set at 324 nm. The extinction was continuously recorded. All ointment components were checked for any interferences and none of them give an absorbance at 324 nm.

In-vivo bioavailability study

Six white male rabbits (Witte van Dendermonde), weighing 3 kg were utilized for each experiment. Four different ointments, each of them containing 2.5 % metronidazole, were chosen for the in-vivo experiments : the polyethylene glycol ointment, the emulsion base (B_2), the Carbopol 940 gel and the oleaginous ointment. The rabbit's skin was shaved and treated with a depilatory 24 hr before the experiment. An area of 7.5 x 15 cm was covered with ointment (± 8 g), the animal was restrained in a wooden rabbit holder, exposing only the head. 1 ml blood samples were taken from the marginal ear vein 45, 60, 90, 120 and 180 min after dosing. A predose blood sample to serve as a blank was taken 15 min before administrating the drug.

Plasma samples were prepared and kept at -25 °C prior to analysis.

Analytical procedure

A HPLC method developped by Jensen and Gugler (1983) was used for both stability determination and bioavailability experiments. The system included a HPLC pump (Waters Model 5000 A, Milford, MA, USA), a reversed phase column (microBondapack 15 cm x 4.6 mm - average particle size 5 µm, Alltech RSL, Eke, Belgium) and UV detector (Pye Unicam Model L.C. 3, Cambridge, England) set at 324 nm and an injector (Waters Universal Injector Model U 6 K, Milford, MA, USA).

The mobile phase consisted of 0.005 M monopotassium phosphate-methanol-tetrahydrofuraan (82.6:16.5:0.9) mixture. The operating flow rate was 1.4 $ml.min^{-1}$ and the temperature ambiant.

Sample preparation for the stability determination

A 0.100 gram ointment sample was weighed, for each ointment analysis, in 10 ml glass tubes and 5 ml water added. The mixture was vortexed for 30 s. The test tubes were put in a constant temperature water bath at 80 °C during 15 min. Every 5 min the mixture was shaken. Next the tubes were centrifuged for 15 min at 2500 rpm. Depending on the original metronidazole concentration in the ointments (0.5 %, 1 % or 2.5 %), 1 ml, 0.5 ml or 0.2 ml of the aqueous phase were transferred to another 10 ml test tube. To each of the tubes 100 µλ internal standard solution (aqueous tinidazole solution containing 100 mg/100 ml) were pipetted. Finally the volume was completed to 10.0 ml with water. A 200 µλ portion was injected in the chromatograph.

A calibration curve was prepared, using known concentrations of metronidazole in the ointment, by plotting the concentration (µg/ml) against peak height ratios. The curve was linear over a 0-15 µg/ml concentration range ($y = 4.64x - 0.015$; $r^2 = 0.99989$).

Plasma Assay

A 1 ml plasma sample, 250 µλ ethanolic tinidazole solution (100 mg/100 ml) as the I.S. and 250 µλ zinc sulphate solution (0.1 M) are transferred to 10 ml glass stoppered tubes. The mixture is vortexed for 120 s., kept at 4 °C during 15 min and centrifuged at 3000 rpm during 5 min. A 20 µλ portion was injected in the chromatograph. A calibration curve was prepared using known metronidazole concentrations with plasma, plotting the concentration (µg/ml) against the peak height ratio.

The curve was linear over a 0-8 µg/ml concentration range ($y = 0.1973x - 0.0044$; $r^2 : 0.99999$).

RESULTS AND DISCUSSION

Stability study

Different ointment formulations at concentrations of 0.5 %, 1 % and 2.5 % have been stored during 9 month at 40 °C and 4 °C. All of the formulas tested show excellent stability independent of drug concentration or ointment base. The emulsion base prepared with 1 % lactic acid showed a brownish discolouration at both 4 °C and 40 °C and the metronidazole content was reduced to 68.5 % after 3 month storage at 40 °C. This indicates that an acidic pH, in casu 2.25, has a deleterius effect on the drug stability.

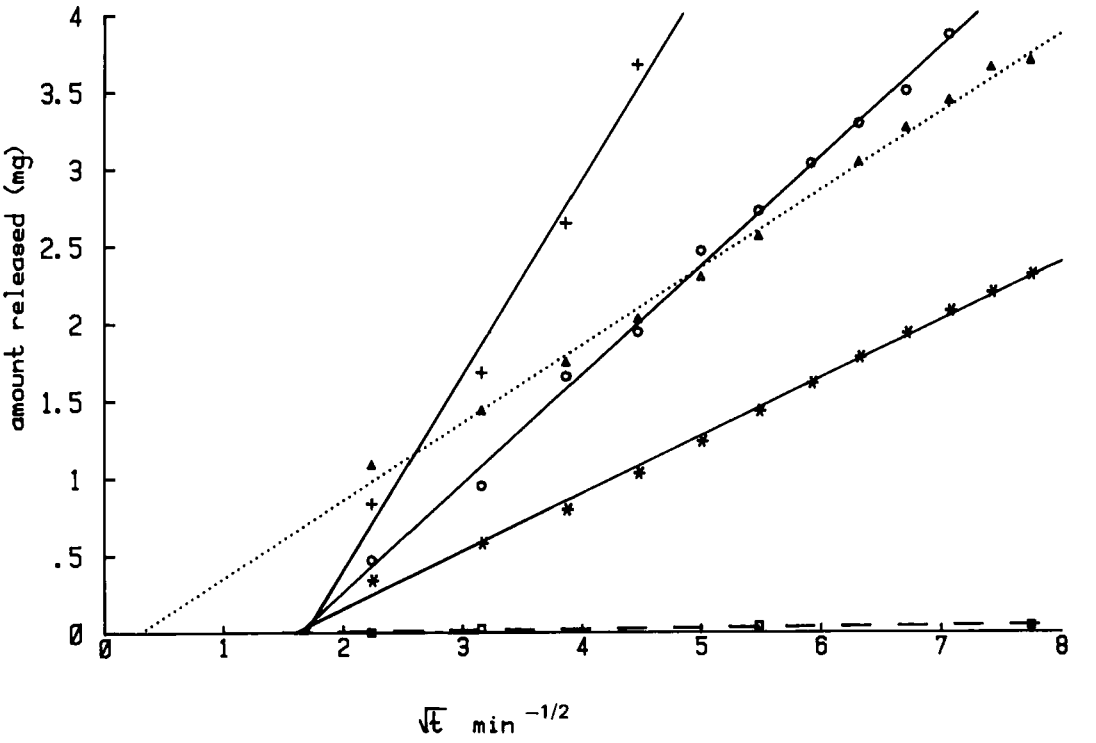

FIGURE 1

Influence of ointment base composition on the release of metronidazole from ointments containing 2.5 % (W/W) drug. Each curve is the mean of six determinations. Key : -+- polyethylene glycol ointment; ...Δ... Carbopol gel; -o- emulsion base (B_2); -*- emulsion base (B_1) and --□-- oleaginous base.

In-vitro release

Figure 1 gives the release characteristics of metronidazole (1 %) from the different ointment bases over an one hour period. In all cases, linear plots were obtained when amount of metronidazole released was plotted versus the square root of time. In no cases did the plots pass through the origin. This short lag time (< 5 min) can be explained as the time required for the absorption of drug by membrane.

The rate of release decreases in the following order : polyethylene glycol, emulsion O/W (type B_2), Carbopol gel, emulsion O/W (type B_1), oleaginous base. The solution type ointment (polyethylene glycol ointment) shows the highest release rate compared to the other suspension ointments. The very rapid release of metronidazole from the macrogol ointment is due to a very low affinity of metronidazole for polyethylene glycol. Expressed as the amount of metronidazole released, the curves indicate that 18 % was released from polyethylene glycol ointment after 20 min, 10 % from the emulsion type ointment (B_2), 17 % from the hydrogel but only 0.21 % from the oleaginous base after 50 min. For the emulsion and hydrogel ointments, where metronidazole was suspended in the base and where differences in solubility are not the overwhelming factor, water being the external phase, the diffusity of the drug in the base may have a significant influence on the release characteristics of the drug. The very slow solubility of metronidazole in the lipophilic base can explain the low resorption rate of suspended metronidazole.

In-vivo study

The mean plasma metronidazole concentration-time profiles obtained from six individual rabbits after application of different ointment containing 2.5 % (W/W) drug are shown in Figure 2. As can be seen from the data in Table 2, the maximum plasma concentration (C_p^{max}) obtained show a significant difference (Kruskal-Wallis test $p < 0.01$) for respectively the oleaginous base, the Carbopol gel, the emulsion type ointment (B_2) and the polyethylene glycol ointment. The time of

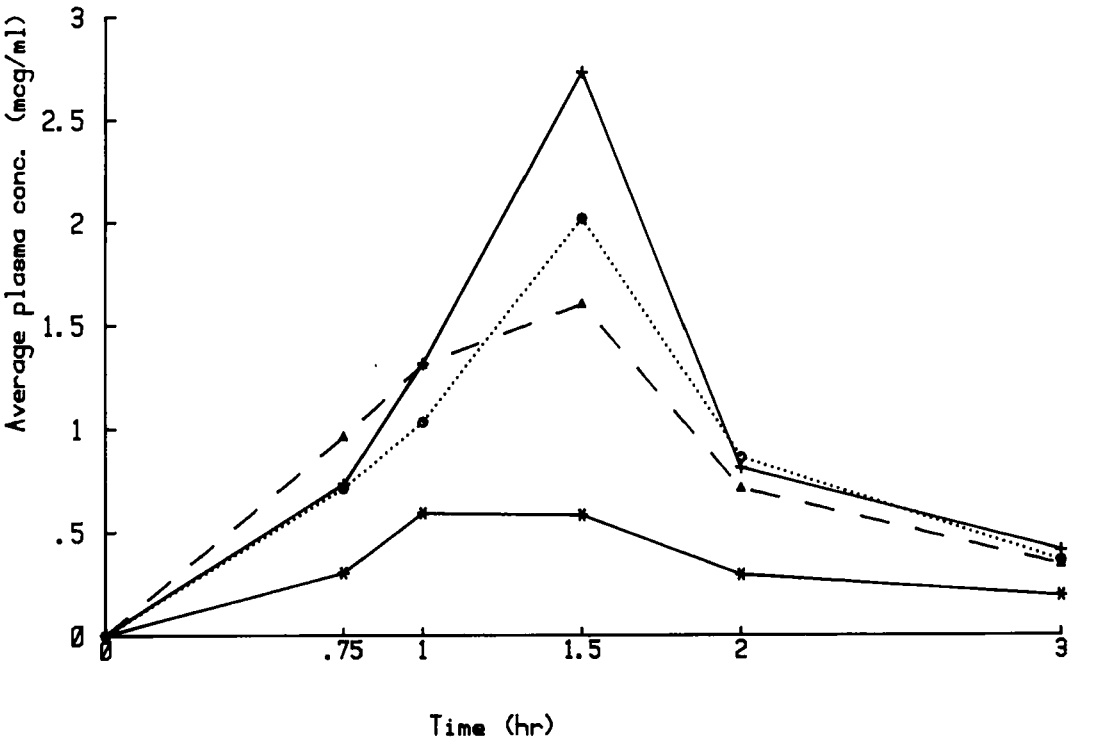

FIGURE 2

Average plasma metronidazole concentration (mcg/ml) obtained from six rabbits after topical application of metronidazole ointments.
Key : -+- polyethylene glycol ointment; ...o... emulsion ointment (B_2); ---Δ--- Carbopol gel and -✱- oleaginous ointments.

occurence of the maximum peak level was the same for all bases with the exception of the oleaginous base where a plateau rather than a maximum, was observed.

There is a significant difference between the area under the curve for the several ointments tested (Kruskal-Wallis test $p < 0.02$). The mean area under the curve reached from 1.85 $\mu g.ml^{-1}h^{-1}$ for the lipophilic

TABLE 2

Average plasma concentration for each time point Maximal plasma concentration (C_p^{max}), time to reach C_p^{max} (t_{max}) and area under the curve (A.U.C.) after topical administration of four different metronidazole ointments (2.5 % W/W). Each ointment was administered to six individual rabbits.

BASIS	OLEAGINOUS		CARBOPOL GEL		EMULSION BASE (TYPE B_2)		POLYETHYLENE GLYCOL BASE	
Time(h)	µg/ml	(±S.E.)	µg/ml	(±S.E.)	µg/ml	(±S.E.)	µg/ml	(± S.E.)
0.75	0.30	0.26	0.96	0.61	0.71	0.49	0.73	0.43
1	0.59	0.38	1.308	0.60	1.03	0.73	1.31	0.86
1.5	0.58	0.29	1.603	0.48	2.02	0.52	2.73	0.47
2	0.29	0.30	0.71	0.39	0.86	0.49	0.81	0.44
3	0.19	0.16	0.34	0.29	0.36	0.23	0.41	0.19
C_p^{max} * (µg/ml)	0.59	(±0.38)	1.60	(±0.48)	2.02	(±0.52)	2.73	(±0.48)
t_{max}(h)	-	-	1.5	-	1.5	-	1.5	-
A.U.C.** (µg/mlxh)	1.85	(±1.43)	4.99	(±2.11)	5.07	(±2.50)	6.58	(±2.53)

* : Kruskal-Wallis test $p < 0.001$

** : Kruskal-Wallis test $p < 0.02$

ointment to 6.58 $\mu g.ml^{-1}h^{-1}$ for the polyethylene glycol ointment. Carbopol gel revealed a value of 4.99 $\mu g.ml^{-1}h^{-1}$ in comparison with 5.07 $\mu g.ml^{-1}h^{-1}$ for the emulsion base.

So it is apparent that metronidazole was released better from a polyethylene glycol base than from other bases and this is in agreement with the in-vitro data.

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