

## Influence of the formulation type (o/w, w/o/w emulsions and ointment) on the topical delivery of paromomycin

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*Ointments containing paromomycin (PA) have been used for topical treatment of cutaneous leishmaniasis. Although influence of the vehicle on skin permeation is crucial for optimization of formulations containing PA, this aspect has not been investigated experimentally. In this study, we have evaluated the influence of the formulation type [oil-in-water (o/w), water-in-oil-in-water (w/o/w) emulsions and ointment] on in vitro release and skin permeation of PA and experiments were carried out on cellulose acetate membrane and skin biopsies from hairless mice, respectively. PA release from o/w emulsion ( $51.7\% \pm 0.9$  of dose applied) was faster than that observed for w/o/w emulsion ( $3.0\% \pm 0.5$ ). PA absorption from o/w emulsion ( $87.1\% \pm 3.9$ ) through stripped skin was 5 to 6 times higher than that observed for w/o/w multiple emulsion ( $14.7\% \pm 0.5$ ) and ointment ( $16.6\% \pm 0.1$ ). PA permeation through intact (normal) skin was negligible for all tested formulations. Skin uptake from o/w emulsion ( $0.9\% \pm 0.1$ ) was greater than that observed for w/o/w multiple emulsion ( $0.3\% \pm 0.1$ ) and ointment ( $0.1\% \pm 0.1$ ). These results suggest that a hydrophilic vehicle (o/w emulsion) could be an interesting alternative to improve topical delivery of PA.*

### Uniterms:

- Paromomycin
- Topical delivery
- Emulsions
- Ointments
- Cutaneous leishmaniasis

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## INTRODUCTION

Tegumentary leishmaniasis is a complex group of diseases caused by various different *Leishmania* species. Each species is responsible for a certain group of clinical symptoms, which can vary from the presence of single localized ulcers (cutaneous leishmaniasis) to the diffuse or the disfiguring mucocutaneous forms of the disease (Barral-Neto *et al.*, 1995).

Currently, pentavalent antimonials, administered parenterally, are considered the first line drugs in treatment

of cutaneous leishmaniasis. This treatment causes a variety of side effects, including changes in the electrocardiogram and increase of hepatic transaminases. Thus, many alternative systemic and topical treatments have been used to treat cutaneous leishmaniasis (Grevelink, Lerner, 1996).

As an alternative treatment, local therapy can be of value because of its simple administration, safety and fewer side effects. Paromomycin sulphate (PA), a aminoglycoside antibiotic, has demonstrated a high *in vitro* activity against *Leishmania major* amastigotes in

macrophages (El-On, Greenblat, 1983; Neal *et al.*, 1995).

In experimental models and clinical studies, El-On *et al.* (1984, 1986, 1988) have been demonstrated that an ointment containing 15% PA and 12% methylbenzethonium chloride (MBCL), a quaternary ammonium surfactant, was effective against *L. major*. The local side effects due to MBCL (Berman, 1997) have led to development of a formulation containing PA and urea as skin penetration enhancer of the drug (Neal *et al.*, 1994). This ointment cured all *L. major* lesions on Balb/C mice, however clinical trials carried out in Tunisia (Salah *et al.*, 1995) and in Iran (Asilian *et al.*, 1995) suggest that this formulation was not as effective as that containing PA/MBCL. The modification of the vehicle (base) could be also an alternative approach to enhance the anti-leishmanial activity of PA.

Although it is well established that the vehicle greatly influences drug percutaneous absorption, this aspect has not been experimentally investigated for the topical application of PA. The vehicle can influence drug absorption both by changing the stratum corneum permeability or the thermodynamic activity of the drug (Moser *et al.*, 2001; Lalor, Flynn, Weiner, 1994, 1995). Thus, the aim of this study was to investigate, by the first time, the influence of the vehicle on *in vitro* release and skin permeation of PA from emulsions and an ointment.

Since the cutaneous leishmaniasis lesions often develop into an ulcer without stratum corneum (Grevelink, Lerner, 1996; Neal *et al.*, 1994), the principal barrier for skin penetration, a vehicle-controlled delivery system (w/o/w multiple emulsion) containing PA was also developed. The skin permeation and release of glucose, as hydrophilic drug model, from w/o/w emulsion was prolonged when compared with that obtained with an o/w emulsion (Ferreira *et al.*, 1995). Thus, the three preparations (o/w and w/o/w emulsions and ointment) were compared.

## MATERIAL AND METHODS

### Material

Paromomycin sulfate (PA), potency of 718 mg/g, was donated by Pharmacia & Upjohn (Milan, Italy). The surfactants used were: a silicone-based polymeric lipophilic surfactant (Abil EM90<sup>®</sup>; HLB=3) obtained from Goldschmidt (France) and a hydrophilic surfactant, a block copolymer of ethylene and propylene oxide (Synperonic PEF/127<sup>®</sup>; HLB=18-23) donated by ICI Surfactants (Belgium). Paraffin oil and soft white paraffin were obtained from Favab (Brazil). All other chemicals used were analytical reagent grade.

### Methods

#### *Preparation of the formulations*

In order to investigate only the influence of the emulsion type, w/o/w multiple and o/w simple emulsions were prepared according to the same formula. It was similar to that described previously (Ferreira *et al.*, 1994, 1995): 22% of paraffin oil, 3% of lipophilic surfactant, 1% of hydrophilic surfactant, 1% of paromomycin sulfate (PA) and 73% of 0.08 M NaCl, by weight. However, procedures of emulsification were different.

W/o/w multiple emulsion was prepared by a two-stage emulsification procedure. In the first step, a w/o primary emulsion was formed by adding the aqueous phase to oily phase at 8,000 rpm for 30 minutes. Emulsification was carried out using a ULTRA TURRAX T-25 type agitator (IKA, Staufen, Germany). In a second step, w/o primary emulsion was dispersed to external aqueous phase using an EUROSTAR basic agitator (IKA, Staufen, Germany) at 400 rpm for 15 minutes (Table I).

O/w emulsion was prepared through the addition of the aqueous phase to oily phase at 8,000 rpm for 15

**TABLE I** - Composition (% w/w) of the w/o/w multiple emulsion

Steps	Phase	Components	% w/w
<b>First</b> w/o emulsion	Oily	Oil <sup>a</sup>	30
		Hydrophobic surfactant <sup>b</sup>	4
	inner aqueous	NaCl 0.08M	64.7
		Paromomycin	1.3
<b>Second</b> w/o/w emulsion	external aqueous	w/o emulsion	75
		Hydrophilic surfactant <sup>c</sup>	25

<sup>a</sup>Paraffin oil; <sup>b</sup>a silicone-based polymeric hydrophobic surfactant (Abil EM90<sup>®</sup>; HLB=3); <sup>c</sup>a block copolymer of ethylene and propylene oxide (Synperonic PEF/127<sup>®</sup>; HLB=18-23).

minutes. Emulsification was carried out using an ULTRA TURRAX T-25® agitator at 25 °C. Ointment was prepared by triturating PA into soft white paraffin until a homogeneous mixture has been obtained.

#### *Characterization of the emulsions*

After preparation, microscopic observations (Olympus-Micronal, Brazil) and conductivity values (Conductivity Meter Micronal B-330, Brazil) confirmed that two emulsion types (w/o/w or o/w) were obtained.

Percentage of PA entrapped in internal phase of the w/o/w emulsion was determined through the difference between total and encapsulated PA content. To determine total PA content, samples of 100 mg of the multiple emulsions were diluted with 100 mL of distilled water and extracted with three 40-mL quantities of chloroform. Chloroform was discarded and PA concentration in aqueous solution was determined by microbiologic assay (see below). Not encapsulated PA content was determined after dilution of samples (100 mg) in 25 mL of an aqueous solution of glucose at same osmolarity as that of the internal aqueous phase. Aliquots were filtered through Millipore filter (0.45 mm) and PA concentration was determined by microbiologic assay.

#### *In vitro release of PA*

*In vitro* release of PA was determined in modified Franz diffusion cells, as described previously (Ferreira *et al.*, 1995). Briefly, cellulose acetate membranes (thickness: 25 ± 1 mm; MWC 5.000; Dianorm - Munique) were placed horizontally dividing the cell in two compartments: the donor and the receptor. Receptor compartment was filled with 0.05 M phosphate buffered (pH 7.4) saline. This receptor liquid, maintained at 37 °C, was continually stirred with a small magnetic bar.

These experiments were carried out in infinite dose and the donor compartment was covered with Parafilm® (American National Can, Greenwich, CT). Preparations were applied (500 mg) and serial sampling was performed at specific times by totally removing receptor fluid and refilling with fresh solution. PA concentration was determined by microbiologic assay (see below).

#### *In vitro skin permeation*

*In vitro* skin permeation through dorsal skin excised from hairless mice was determined with the same Franz cells described above. Male, 8 weeks old hairless mice (strain HRS/J Jackson Laboratories, Bar Harbor, ME) were sacrificed by cervical rupture and the dorsal skin was used immediately. These experiments were made in the presence or absence of the stratum corneum (SC). SC was

removed by stripping 5 times with a cellophane tape (SCOTH Magic n° 810, 3M). Receptor compartment was filled with 0.05M phosphate buffered saline (pH 7.4).

Donor compartment was left open and preparations (50 mg), containing 1% w/w of PA, were applied on the skin and spread with a glass rod. Serial sampling was performed as described above.

At the end of the experiments, removal of the excess formulation was determined by washing and rinsing of the treated surface twice times with 500 µL of liquid cleansing soap at 5% (Extrán®, Merk) and twice times with 500 µL of water, removing the residue with a cotton swab. Washing solvent, pipette tips and cotton swab were added to a bottle containing 100 mL of distilled water. PA concentration in this solution was determined by microbiologic assay. Thus, recovery from ointment was of 95.5% ± 2.2 of the dose applied. PA remaining into skin (dermis + epidermis) was extracted as follows: skin biopsies were vortex-mixed for 15 minutes in distilled water (2 mL); samples were centrifuged and supernatant fractions were filtered through Millipore filter (0.45 mm). PA concentration was determined by microbiologic assay. Formulations without PA were also submitted to all protocols described previously. These samples did not interfere with the analysis method.

#### *Microbiologic assay of the PA*

Microbiological assays are traditionally used for determination of aminoglycoside antibiotics. Assay of PA was made according methodology described in United States Pharmacopoeia (USP 23, 1995). Briefly, concentration of PA was determined by an agar-diffusion inhibition assay of the growth of a test organism, *Staphylococcus epidermidis* (ATCC 12,228). Diffusion of PA in agar resulted in an inhibition halo that represented the absence of growth of the microorganism. A linear relationship between diameter of the inhibition halo and logarithm of the PA concentration was observed. PA concentration was determined from a standard curve ( $y = 9.9417x + 8.5568$ ;  $R^2 = 0.9988$ ).

#### **Statistical Analysis**

Analysis of variance (ANOVA) was used to evaluate differences between means. Differences at level  $p < 0.05$  were considered significant.

## **RESULTS AND DISCUSSION**

The aim of this study was to evaluate the influence of the vehicle on topical delivery of PA. Therefore,

membrane of cellulose acetate was used only as a preliminary step to studies that will include *in vitro* skin permeation. This synthetic membrane has been used as *in vitro* release method for creams, ointments and gels based on the principle that the effectiveness of topical products is dependent on the release of active drug from its dosage form (Shah, Skelly, 1993).

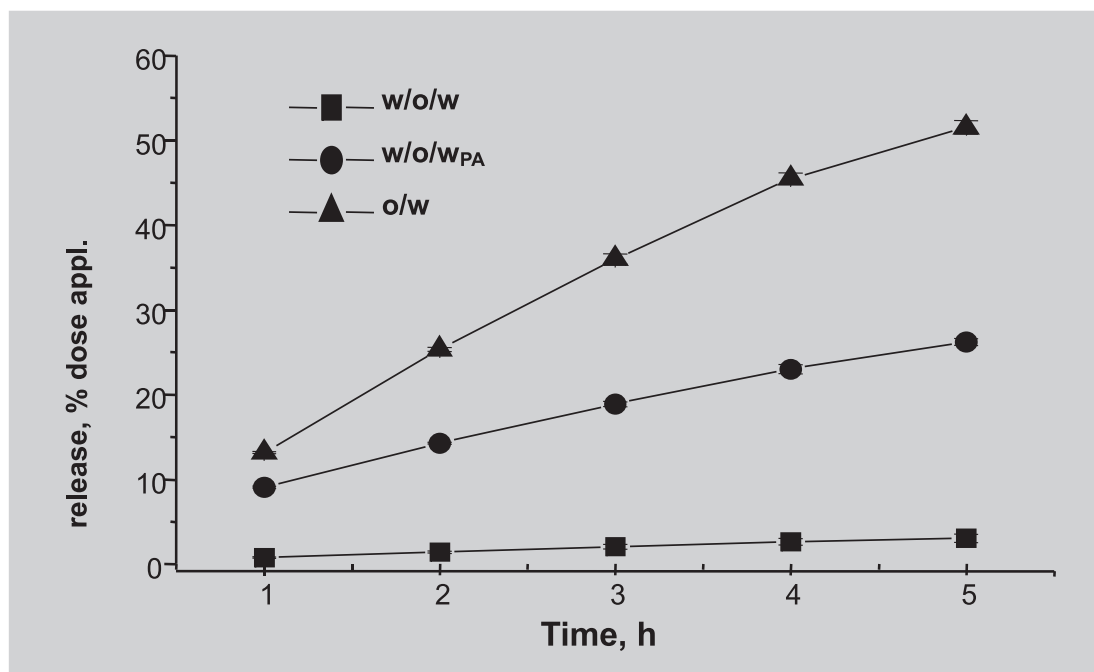
Release of PA from o/w simple emulsion ( $51.7\% \pm 0.9$  of the applied dose) was considerably greater than that observed from w/o/w multiple emulsion ( $3.0\% \pm 0.5$ ). When half of the amount of PA was added to external aqueous phase of the w/o/w multiple emulsion (w/o/w<sub>PA</sub>), released amount was intermediary ( $26.2\% \pm 0.4$ ) in comparison with other preparations (Figure 1).

High release from o/w emulsion can be attributed to availability of PA in aqueous phase of this emulsion. Lalor, Flynn, Weiner (1994, 1995) showed that, in an emulsion, interaction of the drug between water and oil phase can determine extent of lowering of the thermodynamic activity in external phase which is in contact with membrane. Partitioning of PA into internal oily phase or its micellar solubilization in external phase of the o/w emulsion is unlikely, due to hydrophilic characteristic of the drug.

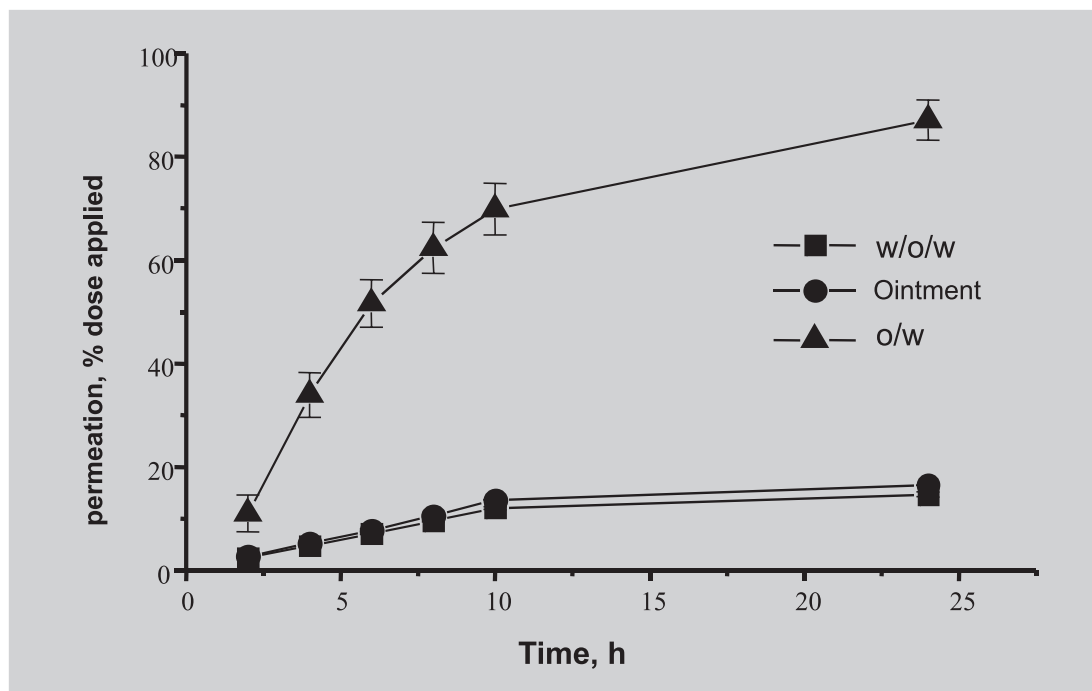
The extremely slow release of PA from the multiple emulsion can be explained by the fact that diffusion of PA through oily phase is the limiting step for drug release.

Percentage of PA entrapped in internal aqueous phase of the W/O/W emulsion was high (91.3%), whereas the PA percentage in external aqueous phase was low (8.7%). Only this content of PA in external phase would be immediately available for release. A similar phenomenon has been observed for glucose from multiple emulsion (Ferreira *et al.*, 1995). This hypothesis is also supported by the fact that PA release from w/o/w<sub>PA</sub> was greater than that observed from w/o/w multiple emulsion, once that a larger amount of drug is available in external phase.

In order to validate PA recovery from surface of the skin, previous experiments, performed immediately after the application of the preparations, were conducted. The data showed that PA recovery from o/w, w/o/w emulsions and ointment was of  $95.1\% \pm 2.5$ ,  $92.9\% \pm 1.8$  and  $62.8\% \pm 1.1$  (n=3), respectively. In order to improve PA recovery rate from lipophilic ointment, a first washing was performed with 500  $\mu$ L of chloroform and then as described for the other formulations (Extran<sup>a</sup> and water). Skin uptake and permeation data of PA from emulsions and ointment through stripped skin are redrawn in Table II and Figure 2, respectively. Mass balance ranged from 92 to 98%. Skin permeation of PA from o/w emulsion ( $87.1\% \pm 3.9$ ) was 5 times higher than that observed for w/o/w multiple emulsion ( $14.7\% \pm 0.5$ ) and ointment ( $16.6\% \pm 0.1$ ). Greater efficacy of the o/w emulsion for delivering



**FIGURE 1** - *In vitro* release of PA from o/w, w/o/w and w/o/w<sub>PA</sub> emulsions across cellulose acetate membrane. The w/o/w<sub>PA</sub> multiple emulsion contains half of the PA amount into external aqueous phase. The formulations containing 1% w/w of PA were applied (500 mg-infinite dose) over synthetic membranes mounted in Franz cells. The expressed values correspond to the mean value  $\pm$  SD (n=3).



**FIGURE 2** - *In vitro* skin permeation of PA from o/w, w/o/w emulsions and ointment through dorsal skin excised from hairless mice in partial absence of *stratum corneum*. The formulations containing 1% w/w of PA were applied (50 mg-finite dose) over skin biopsies mounted in Franz cells. Each point represents average ( $n=3$ )  $\pm$  SD.

PA might be due to higher concentration of the drug in external phase and/or to rapid increase in thermodynamic activity due to evaporation of water. Changes in thermodynamic activity of the drug, through evaporation of a volatile vehicle, can often be a significant factor in topical delivery (Chiang *et al.*, 1989; Tata, Flynn, Weiner, 1995).

There is a good correlation between the permeation and release data for the o/w emulsion and multiple emulsion. Therefore, release and skin permeation of PA from o/w emulsion were higher than those observed for multiple emulsion, despite of differences in experimental protocols (infinite and finite dose).

An analysis of Figure 2 demonstrates that flux of PA from w/o/w emulsion and ointment already declines when only 14 to 16% of the applied dose has been permeated.

For the multiple emulsion, this phenomenon could be attributed to depletion of PA in the external aqueous phase. For the ointment, this can be explained by permeation of a small fraction of PA which was initially solubilized in this preparation. These results suggest that the release of a small fraction of PA from both formulations was associated to a low skin penetration.

Since the skin permeation was greater from o/w emulsion in comparison with other preparations, a higher concentration of PA into skin could be expected. However, surprisingly, PA uptake into stripped skin from o/w emulsion was lower than that observed for w/o/w emulsion (Table II). This can be explained by high depletion of the PA concentration in donor compartment, once 87% of the applied dose have been permeated from o/w emulsion after 24 hours.

**TABLE II** - Distribution<sup>a</sup> of PA (% dose applied), into stripped skin, after 24 hr

Formulations	Permeation	Skin <sup>b</sup>	Surface wash	Total recovery(%)
o/w emulsion	87.1 $\pm$ 3.9	1.1 $\pm$ 0.1	9.6 $\pm$ 1.7	97.9
w/o/w emulsion	14.7 $\pm$ 0.5	1.7 $\pm$ 0.2	75.2 $\pm$ 1.9	91.5
Ointment	16.6 $\pm$ 0.1	0.9 $\pm$ 0.1	74.3 $\pm$ 2.7	91.7

<sup>a</sup>formulations containing 1% w/w of PA were applied (50 mg-finite dose) over stripped skin biopsies from hairless mice mounted in Franz diffusion cells; The SC was removed by stripping 5 times the skin; <sup>b</sup>epidermis + dermis; the expressed values correspond to the mean value  $\pm$  SD ( $n=3$ ).

**TABLE III** - Distribution<sup>a</sup> of PA (% dose applied), into normal skin, 24 hr after application of the formulations

Formulations	Permeation	Skin <sup>b</sup>	Surface wash	Total recovery(%)
o/w emulsion	ND <sup>c</sup>	0.9 ± 0.1	99.5 ± 2.6	100.4
w/o/w emulsion	ND	0.3 ± 0.1	91.0 ± 1.7	91.3
Ointment	ND	0.1 ± 0.1	98.5 ± 7.7	99.1

<sup>a</sup>formulations containing 1% w/w of PA were applied (50 mg-finite dose) over intact skin biopsies from hairless mice mounted in Franz diffusion cells; <sup>b</sup>epidermis + dermis; <sup>c</sup>no detected.

The expressed values correspond to the mean value ± SD (n=3).

Skin uptake and permeation of PA through intact skin from tested preparations are redrawn in Table III. The mass balance ranged from 91 to 100%. PA permeation was not detectable and below of detection limit. As expected, PA permeation through stripped skin was higher than that observed for intact skin. PA uptake into intact skin from o/w emulsion (0.9% ± 0.1) was about 3 and 10 times higher than that observed for w/o/w multiple emulsion (0.3% ± 0.1) and ointment (0.1% ± 0.1), respectively (Table III). Improved topical delivery of PA from o/w emulsion can be attributed to thermodynamic activity higher than that observed for other formulations, as discussed earlier. Total deposition (skin uptake) and skin permeation of lactic acid into or through porcine skin, determined using a finite dose, was in the following order: o/w > w/o/w > w/o emulsions (Sah, Mukherjee, Wickett, 1998).

Although it has not been possible to evaluate skin permeation of PA through the intact skin, our studies conducted with stripped skin, a model that may be closer to what is observed in cutaneous leishmaniasis, showed significant differences among formulations. Our findings are in good agreement with those previously reported (Stone *et al.*, 1968), in which the percutaneous absorption of gentamycin, an antibiotic structurally related to PA, from water-miscible bases was greater and faster than from ointment bases.

In conclusion, release of PA from o/w emulsion was faster than from w/o/w emulsion. PA release from w/o/w<sub>PA</sub> multiple emulsion containing half of the PA amount in the external aqueous phase was intermediate. Skin uptake and permeation of PA from o/w emulsion were higher than those observed for w/o/w emulsion and ointment. An important consideration in cutaneous leishmaniasis is the skin condition. Topical formulations may be applied either to open lesions that have lost SC barrier property or to thickened lesions that represent an additional barrier to absorption (Garnier, Croft, 2002). Thus, our findings suggest that a hydrophilic vehicle (o/w emulsion) could be an interesting alternative to improved topical delivery of PA in both conditions. Additional studies are required to

evaluate the influence of skin permeation enhancers incorporated in this vehicle.

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## RESUMO

### Influência da forma farmacêutica (emulsão múltipla A/O/A, emulsão O/A e pomada) sobre a permeação e retenção cutânea da paromomicina

*Pomadas contendo paromomicina (PA) são utilizadas no tratamento tópico da leishmaniose cutânea. Embora a influência do veículo seja crucial para otimização de formulações contendo PA, este aspecto não tem sido investigado experimentalmente. Neste estudo, nós avaliamos a influência do tipo de formulação [emulsões óleo-em-água (o/a), múltipla água-em-óleo-em-água (a/o/a) e pomadas] sobre a liberação e permeação cutânea in vitro da PA e os experimentos foram conduzidos sobre membranas de acetato de celulose e biópsias de pele de camundongos glabros, respectivamente. A liberação de PA a partir da emulsão o/a (51,7% da dose aplicada) foi mais rápida do que aquela observada a partir da emulsão múltipla a/o/a (3,0% da dose aplicada). A permeação cutânea da PA a partir da emulsão o/a (87,1% ± 3,9), através da pele sem estrato córneo, foi 5 a 6 vezes mais alta do que aquela observada para a emulsão múltipla a/o/a (14,7% ± 0,5) e pomada (16,6% ± 0,1), respectivamente. A permeação da PA através da pele normal foi desprezível para todas as formulações avaliadas. Nesta condição, a retenção cutânea a partir da emulsão o/a (0,9% ± 0,1) foi maior do que aquela*

*observada para a emulsão múltipla (0,3% ± 0.1) e pomada. Esses resultados sugerem que um veículo hidrofílico seria uma alternativa interessante para aumentar o transporte e a permeação cutânea da PA.*

*UNITERMOS: Paromomicina. Permeação cutânea. Emulsões. Pomadas. Leishmaniose cutânea.*

## REFERENCES

- ASILIAN, A.; JALAYER, T.; WHITWORTH, J.A.G.; GHASEMI R.L.; NILFOROOSHZADEH, M.; OLLIARO, P. A randomized, placebo-controlled trial of a two-week regimen of aminosidine (paromomycin) ointment for treatment of cutaneous leishmaniasis in Iran. *Am. J. Trop. Med. Hyg.*, Lawrence, v.53, p.648-651, 1995.
- BARRAL-NETO, M.; MACHADO, P.; BARRAL, A. Human cutaneous leishmaniasis: recent advances in physiopathology and treatment. *Eur. J. Derm.*, Montrouge, v.5, p.104-13, 1995.
- BERMAN, J.D. Human Leishmaniasis: clinical, diagnostic and chemotherapeutic developments in the last 10 years. *Clin. Infect. Dis.*, Chicago, v.24, p.684-703, 1997.
- CHIANG, C.M.; FLYNN, G.L.; WEINER N.D.; SZPUNAR, G.J. Bioavailability assessment of topical delivery systems: effect of vehicle evaporation upon *in vitro* delivery of minoxidil from solution formulations. *Int J. Pharm.*, Amsterdam, v.55, p.229-236, 1989.
- EL-ON, J.; GREENBLATT, C.L. An *in vitro* model for testing the effect of anti-leishmanial drugs of possible use in topical treatment. *Curr. Ther. Res.*, New York, v.33, p.660-669, 1983.
- EL-ON, J.; JACOBS, G.P.; WITZTUM, E.; GREENBLATT, C.L. Development of topical treatment for cutaneous leishmaniasis caused by *Leishmania major* in experimental animals. *Antimicrob. Agents Chem.*, Washington, v.26, p.745-751, 1984.
- EL-ON, J.; LIVSHIN, R.; EVEN-PAZ, Z.; HAMBURGER, D.; WEINRAUCH, L. Topical treatment of cutaneous leishmaniasis. *J. Invest. Dermatol.*, New York, v.87, p.284-288, 1986.
- EL-ON, J.; JACOBS, G.P.; WEINRAUCH, L. Topical chemotherapy of cutaneous leishmaniasis. *Parasitol. Today*, Amsterdam, v.4, p.76-81, 1988.
- FERREIRA, L.A.M.; SEILLER, M.; GROSSIORD, J.L.; MARTY, J.P.; WEPIERRE J. Vehicle influence on *in vitro* release of metronidazole: Role of w/o/w multiple emulsion. *Int. J. Pharm.*, Amsterdam, v.109, p.251-259, 1994.
- FERREIRA, L.A.M.; SEILLER, M.; GROSSIORD, J.L.; MARTY, J.P.; WEPIERRE J. Vehicle influence on *in vitro* release of glucose: w/o, w/o/w and o/w systems compared. *J. Control. Release*, Amsterdam, v.33, p.349-356, 1995.
- GARNIER, T.; CROFT, S.L. Topical treatment for cutaneous leishmaniasis. *Curr. Opin. Investig. Drugs*, London, v.3, p.538-544, 2002.
- GREVELINK, S.A.; LERNER, E.A. Leishmaniasis. *J. Am. Acad. Dermatol.*, St. Louis, v.34, p.257-272, 1996.
- LALOR, C.B.; FLYNN, G.L.; WEINER, N. Formulation factors affecting release of drug from topical formulations. I. Effect of emulsion type upon *in vitro* delivery of ethyl p-aminobenzoate. *J. Pharm. Sci.*, Washington, v.83, p.1525-1528, 1994.
- LALOR, C.B.; FLYNN, G.L.; WEINER, N. Formulation factors affecting release of drug from topical formulations. II. Effect of solubility on *in vitro* delivery of a series of n-alkyl p-aminobenzoates. *J. Pharm. Sci.*, Washington, v.84, p.673-676, 1995.
- MOSER, K.; KRIWET, K.; NAIK, A.; KALIA, Y.N.; GUY R.H. Passive skin penetration enhancement and its quantification *in vitro*. *Eur. J. Pharm. Biopharm.*, Stuttgart, v.52, p.103-112, 2001.
- NEAL, R.A.; ALLEN, S.; COY, N.M.; OLLIARO, P.; CROFT, S.L. The sensitivity of leishmania species to aminosidine. *J. Antimicrob. Chemother.*, London, v.35, p.577-584, 1995.
- NEAL, R.A.; MURPHY, A.G.; OLLIARO, P.; CROFT, S.L. Aminosidine ointments for the treatment experimental cutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.*, London, v.88, p.223-225, 1994.
- TATA, S.; FLYNN, G.L.; WEINER, N.D. Penetration of minoxidil from ethanol/propylene glycol solutions: effect of application volume and occlusion. *J. Pharm. Sci.*, Washington, v.84, p.688-691, 1995.

- SAH, A.; MUKHERJEE, S.; WICKETT, R.R. An *in vitro* study of the effects of formulation variables and product structure on skin permeation of lactic acid. *J Cosmet. Sci.*, v.49, p.257-273, 1998.
- SALAH, A.B.; ZAKRAOUI, H.; ZAATOUR, A.; FTAITI, A.; ZAAFOURI, B.; GARRAOUI, A.; OLLIARO, P.L.; DELLAGI, K.; ISMAIL, R.B. A randomized, placebo-controlled trial in Tunisia treating cutaneous leishmaniasis with paromomycin ointment. *Am. J. Trop. Med. Hyg.*, Lawrence, v.53, p.162-166, 1995.
- SHAH, V.P.; SKELLY, J.P. Practical considerations in developing a quality control (*in vitro* release) procedure for topical drug products. In: SHAH V.P., MAIBACH H.I., eds. *Topical drug bioavailability, bioequivalence and penetration*. New York: Plenum Press, 1993. p. 107-116.
- STONE, H.H.; KOLB, L.D.; PETTIT, J.; SMITH R.B. The systemic absorption of antibiotic from the burned wound surface. *Am. Surg.*, Atlanta, v.34, p.639, 1968.
- UNITED States pharmacopeia. 23.ed. Rockville: United States Pharmacopeial Convention. 1995. p.1690-1696.
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