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Note

Amphotericin B eye drops as a lipidic emulsion

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Abstract

Treatment of ocular mycosis is based on painful instillation of Fungizone® eye drops. The purpose of the study was to develop and to evaluate an Amphotericin B eye drop (AmB) as a better tolerated lipidic emulsion. A concentrated alkaline solution of AmB was mixed with Intralipid® 20%, then neutralized and buffered. The eye drop tolerance and pharmacokinetics were evaluated compared with eye drops in Fungizone® aqueous solution. The tolerance of the AmB lipidic emulsion was better than those observed with a magistral preparation of Fungizone® eye drops ($P < 0.0005$). The average corneal concentrations obtained with the Fungizone® and emulsion eye drops were 501 ± 208 and 274 ± 106 ng/g, repsectively ($P = 0.039$). The average AmB concentrations in the aqueous humour were 490 ± 180 and 300 ± 260 ng/ml, respectively, for the Fungizone® and emulsion eye drops (NS). The plasmatic concentrations measured, lower than 20 ng/ml, were too weak to be toxic.

Keywords: Eye drops; Amphotericin B; Lipidic emulsion; Formulation; Tolerance; Pharmacokinetics

Fungal keratitis are rare infections but extremely serious. The reference treatment remains the Amphotericin B (AmB) eye drops produced as an extemporaneous preparation from the Fungizone® injectable, despite its bad tolerance (O'day et al., 1986). Looking for a new galenical presentation forms an interesting research direction. A galenical study allowed the development of an AmB as a lipidic emulsion, prepared extemporaneously, quickly and simply, in a hospital pharmaceutical department. A pharmacological study evaluated the tolerance and bioavailability of these new eye drops, in order to search for an improvement of ocular tolerance, and/or for an increase of the AmB intraocular penetration.

Eight emulsions with two AmB concentrations (0.5 and 1%) and with two Intralipid 20% (IL 20%) volumes (59 and 77.5%) were prepared (Table 1): AmB was solubilized in sodium hydroxide (NaOH) 0.5 N or N. Fifteen minutes after obtaining a lipidic solution, it was mixed with IL

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Table 1
Composition of various emulsions used (%)

Compound	Emulsion							
	1	2	3	4	5	6	7	8
AmB concentration (m/v)	0	0.5	0	0.5	0	1	0	1
NaOH 0.5 N	19.5	19.5	20	20	0	0	0	0
NaOH N	0	0	0	0	19.5	19.5	19.5	19.5
IL 20%	59	59	77.5	77.5	59	59	77.5	77.5
HCl 0.5 N	19.5	19.5	0	0	0	0	0	0
HCl N	0	0	0	0	19.5	19.5	0	0
HCl 10 N	0	0	qsp pH 7 (0.5)	qsp pH 7 (0.5)	0	0	qsp pH 7 (1)	qsp pH 7 (1)
Phosphate buffer (pH = 7)	2	2	2	2	2	2	2	2
Abbreviations [a]	0–59% (N/2)	0.5–59% (N/2)	0–77.5% (N/2)	0.5–77.5% (N/2)	0–59% (N)	1–59% (N)	0–77.5% (N)	1–77.5% (N)

[a]The first number indicates AmB concentration (m/v) in emulsion, the second, the proportion of 20% IL in this one and the third, the normality of used NaOH for the preparation.

20% then neutralized by hydrochloric acid (HCl) and maintained at pH 7 with the phosphate buffer. Emulsions stored at 4°C away from light were observed at D0 (day of manufacture), D3, D7, D10 and D14. The following tests were performed after distribution into tubes: visual inspection; pH measurement (Orion Research 701 a); osmolality measurement (Fiske Osmometer); granulometric analysis by a photon correlation spectrometer (Nicomp) and by a laser diffraction granulometer (Mastersizer); viscosity and rheological behaviour (Rheomat 30).

This study allowed the choice of the emulsion showing galenical criteria in favour of a better stability. The tolerance and pharmacokinetics of the choosen lipidic emulsion eye drops were evaluated with 12 rabbits after repeated instillations on healthy cornea in comparison with a Fungizone® aqueous solutions. One group of six rabbits was used to study the Fungizone® aqueous solution at 0.5% and the other group of six, the AmB lipidic emulsion at 0.5%. Each rabbit received a 20-μl drop of the tested eye drops in the conjunctival sac in only one eye determined at random. The other eye received a drop of the reference eye drops which had the same composition without AmB. Administrations were repeated every hour for 6 h. Ocular tolerance, carried out after the first and the fifth instillation, was evaluated by a macroscopic observation of the treated eye, a study of the animal behaviour and a cornea study using the slit light. A scale was created for each parameter which allowed to establish a toxicity total score (from 0 to 9) utilized for statistical comparison of the two eye drops. The animals were killed 1 h after the last instillation. Blood (10 μl) was centrifuged and the decanted plasma was frozen at -18°C until dosage. The rabbits' eyes were removed and stored at -18°C for a later dissection (cornea and humour aqueous). The AmB concentrations in the plasma, the cornea and the aqueous humour have been determined by HPLC with spectrometric detection at 410 nm, after pretreatment of biological samples.

The white reference emulsions and IL 20% remained homogeneous during the whole study. In emulsions containing the active compound, the 0.5–77.5% (N/2) emulsion only remained uniformly coloured for 14 days. Other emulsions presented a slight colour gradient or some white areas. The absence of macroscopic phenomena in the emulsions led us to believe that the AmB was

not degraded during the study. With similar emulsion, the molecule remained stable for 50 days (Davis and Washington, 1991). The absence of yellow 'sediment', appearing rapidly when the AmB was incorporated directly into the IL 20% from Fungizone® (Legay-Urvoy et al., 1992), showed the interest of previous solubilization by alkaline solution.

The pH was constant around neutrality, during the study for all emulsions (Table 2). This confirmed the absence of degradation of the active compound that remained stable at neutral pH (Hamilton-Miller, 1973; Hung et al., 1988).

The osmolality differences between emulsions (Table 2) were explained by the more or less important dilution of the IL 20% and by the amount of sodium and chloride ions added. The comparison of AmB containing emulsions and the reference emulsions showed that the molecule did not modify the osmolality. The 1% AmB emulsions with a high osmolality might be badly tolerated by the eye (Ludwig and Van Ooteghem, 1987).

With the photon correlation spectrometer (Table 2), the presence of AmB increased the average diameter in comparison with IL 20% and reference emulsions. The smaller increase in the average diameter of emulsions containing AmB with 77.5% IL 20%, contrary to those containing 59% IL 20%, may have several explanations:—the presence of two droplet populations among which a population corresponded to the IL 20% globules with an average diameter of 358 nm, and the other population resulting from the action of the AmB with a average diameter that could not be detected with this method (>1 μm);—with important volume of IL 20%, the AmB would produce a smaller disturbance of the emulsion characteristics.

Table 2
Average of pH and osmolality (mosm. kg^{-1}.) (average of five measures during 14 days) and average diameter (nm) measured with photon correlation spectrometer (Nicomp)® of eight emulsions and IL 20% at D0 (average of three measures).

Emulsion	pH ± S.D.	Osmolality ± S.D.	Diameter (nm) ± S.D.
IL 20%	8.00 ± 0.00	341 ± 0	340 ± 100
0–59% (N/2)	7.10 ± 0.06	400 ± 6	358 ± 140
0.5–59% (N/2)	7.00 ± 0.06	390 ± 8	519[a] ± 239
0–77.5% (N/2)	7.10 ± 0.06	476 ± 2	366 ± 136
0.5–77.5% (N/2)	7.05 ± 0.04	481 ± 3	395 ± 160
0–59% (N)	7.00 ± 0.05	610 ± 3	345 ± 100
1–59% (N)	7.00 ± 0.05	605 ± 7	588[a] ± 96
0–77.5% (N)	7.00 ± 0.02	672 ± 2	350 ± 150
1–77.5% (N)	7.00 ± 0.06	687 ± 6	388 ± 166

[a]The granulometric distribution of these emulsions is not Gaussian. A non-Gaussian granulometric distribution is an unfavorable criterion for a good emulsion stability.

The distribution of emulsions containing AmB with the smaller proportion of IL 20% (59%) was not Gaussian, showing the instability of a submicronic lipidic emulsion, contrary to emulsions with the higher proportion in IL 20% (77.5%). After D0, the measurements concerning emulsions with AmB could no longer be reproduced.

With the laser diffraction granulometer (Table 3), the presence of AmB and auxiliary substances increased the globule size in comparison with IL 20% which might be due to various phenomena:—an increase in the size of the IL 20% oil globules due to auxiliary solubilizing substances by reducing the electrostatic repulsion between globules;—a flocculation of small globules linked together by the AmB molecules;—a formation of dispersed AmB units with free lecithin molecules present in the IL 20% aqueous phase.

The reference emulsions had the same apparent viscosities and rheological behaviours as IL 20% of 2 mPa.s. Adding AmB was therefore responsible for losing the Newtonian behaviour. The hypothesis previously formulated concerning the globule modification after adding AmB directly applied to viscosity. With agitation, indeed, the large globules might be broken on association with small globules which might be disagregated; these phenomena reduced viscosity (Moes, 1983; Yalabik-kas, 1985). They could be reversible, but more slowly (Zografi, 1982).

According to the results of this study, the choice for animal experimentation was orientated towards the 0.5–77.5% (N/2) emulsion.

Table 3
Evolution of average diameter, of particle percentage inferior to 1 and 5.29 μm of eight emulsions and 20% IL during 14 days with laser diffraction granulometer (Mastersizer)

Emulsion	Average diameter (μm)					% of particles <1 μm					% of particles <5.29 μm				
	D0	D3	D7	D10	D14	D0	D3	D7	D10	D14	D0	D3	D7	D10	D14
IL 20%	0.45	0.45	0.45	0.45	0.45	98.26	98.26	98.26	98.26	98.26	100	100	100	100	100
0–59% (N/2)	0.52	0.51	0.47	0.8	0.46	94.40	95.21	96.32	95.62	97.15	99.96	99.98	99.98	99.97	99.99
0.5–59% (N/2)	2.58	4.72	4.52	4.76	5.31	5.08	0.06	3.60	14.69	0.00	96.94	69.86	72.58	59.12	57.12
0–77.5% (N/2)	0.63	0.52	0.51	0.53	0.49	90.04	93.21	94.56	95.12	95.96	99.41	99.97	99.65	99.95	99.98
0.5–77.5% (N/2)	1.83	5.47	4.32	3.74	3.38	31.48	16.76	6.59	15.69	0.04	98.85	48.73	71.18	77.60	93.47
0–59% (N)	1.03	0.78	0.69	0.70	0.70	62.15	34.43	85.65	86.23	87.30	99.78	96.95	99.72	99.54	99.21
1–59% (N)	0.90	1.19	6.25	4.85	4.29	76.59	61.33	29.28	24.00	0.08	99.52	98.63	50.48	56.14	77.89
0–77.5 (N)	0.67	0.65	0.64	0.65	0.60	85.91	88.95	87.85	89.65	91.31	99.82	99.80	99.82	99.79	99.78
1–77.5 (N)	1.04	1.39	8.42	2.42	2.96	78.09	71.90	43.49	33.0	14.86	97.33	92.80	54.78	92.57	91.05

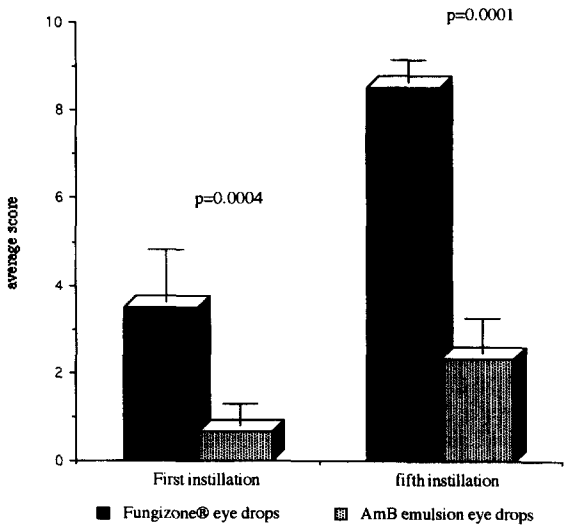

Fig. 1. Ophthalmic tolerance of AmB eye drops. (The smaller the average global, the better the ocular tolerance was).

The tolerance of the Fungizone® eye drops (Fig. 1) decreased in the course of instillations. On the contrary, eye drops as emulsion showed a very good tolerance, even after the fifth instillation. The difference between both groups proved highly significant ($P < 0.0005$). The AmB, described as responsible for a corneal toxicity (Burstein and Klyce, 1977) might not be the only factor questioned here, since it was present in both forms and in same quantity. The presence of the sodium deoxycholate, allowing a micellar solubilization of the AmB, might explain the poor acute or chronic tolerance of the Fungizone® eye drops. Indeed the sodium deoxycholate brought about corneal lesions, more or less serious, depending on the dose received (Adenis et al., 1981; Foster et al., 1958). Thus, producing an emulsion AmB eye drops, without deoxycholate, well tolerated by the animal would allow better compliance with the treatment, possibly leading to better efficiency. This confirmed that in the human being, instillation of IL 10% into the dry eye syndrome was well tolerated and did not cause any discomfort for 30 s after instillation (Rieger, 1990).

The plasmatic concentrations measured below 20 ng/ml were too weak to lead to undesirable systemic effects.

The intraocular penetration of the AmB in an emulsion was not improved in comparison with a same concentration aqueous solution. The average concentration for the Fungizone® group was indeed higher than the emulsion group, not significant in the aqueous humour (490 ± 180 ng/ml for Fungizone® and 300 ± 260 ng/ml for AmB emulsion) and slightly significant ($P = 0.039$) in the cornea (501 ± 208 ng/g for Fungizone® and 274 ± 106 ng/g for AmB emulsions). The presence of sodium deoxycholate in the Fungizone® might explain this difference. This substance is also an absorption promoter (Adenis et al., 1981; Foster et al., 1958). The sodium deoxycholate brought about lesions in the cornea whose seriousness could increase with the number of instillations, thus facilitating the passage of the AmB into the ocular tissues. Besides, the emulsion eye drops could show higher AmB concentrations than aqueous solution eye drops without sodium deoxycholate. The minimal inhibitory concentrations (MIC) of the main germs found in endophtalmies is lower than or around 0.2 μg/ml (Fisher et al., 1983; O'day et al., 1983). Thus, both groups showed an average concentration higher than the MIC of these germs. Our penetration tests concerned healthy rabbit cornea. In the case of a keratitis, the characteristics of the molecule passage are strongly modified: the penetration of all drugs is increased (Adenis and Franco, 1987; Green et al., 1965; Lesar and Fiscella, 1985; O'day et al., 1984). After this necessary experiment on healthy corneas, it would be interesting to test these eye drops on animals with experimental fungal keratitis to judge their clinical efficiency.

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