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Ocular tolerance of sertaconazole gel

Augenverträglichkeit von Sertaconazol-Gel

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Schlüsselwörter. Antimykotische Chemotherapie, Azole, Sertaconazol, Augenverträglichkeit, topische Anwendung, Augenverträglichkeitstest *in vitro*, Draize-Test.

Summary. The *in vitro* and *in vivo* tolerance of sertaconazole gel, a new topical azole antifungal, was studied. Ketoconazole gel (Panfungol®) was used as a reference substance. The methods applied for tolerance assessment were the bovine corneal opacity and permeability test for the *in vitro* assay and a modified Draize test for the *in vivo* assay. The results obtained show that both substances can be classified as slightly irritant and with acceptable tolerance. However, unlike ketoconazole gel, sertaconazole gel did not cause a positive lesion index *in vivo*. Ketoconazole was 5.25 times more irritant *in vitro* than sertaconazole gel, whose effect was similar to that of saline solution. Consequently, the negligible irritant effect of sertaconazole gel on a type of epithelium that is extremely sensitive, i.e. the cornea, confirms the good tolerance of this new antifungal gel on other structures such as the skin and mucous membranes.

Zusammenfassung. Die Toleranz für Sertaconazol-Gel, einem neuen topischen Azol-Antimykotikum, wurde *in vitro* und *in vivo* untersucht. Als Vergleichssubstanz diente Ketoconazol-Gel (Panfungol®). Für den Toleranztest wurden folgende Methoden eingesetzt: Opazitäts- und Permeabilitäts-Test an der bovinen Cornea als *In-vitro*-Versuch sowie der modifizierte Draize-Test als *In-vivo*-Methode. Die Ergebnisse zeigten, daß beide Substanzen als schwach irritierend bei akzeptabler Toleranz einzustufen sind. Im Gegensatz zu Ketoconazol-Gel jedoch ergab sich für Sertaconazol-Gel kein positiver Läsions-Index. Ketoconazol war *in vitro* 5,25 mal so stark irritierend wie Sertaconazol-Gel, dessen Wirkung der von Kochsalzlösung entsprach. Die vernachlässigbare Irritationswirkung von Sertaconazol-Gel auf eines der empfindlichsten Epithelgewebe, der Cornea, gegenüber externen Aggressionen bestätigt daher die gute Verträglichkeit dieses neuen antimykotischen Gels auf andere Gewebe wie Haut und Schleimhäute.

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Introduction

Ocular tolerance is a term used by convention in those studies in which the potential irritant effect of a substance on ocular structures is assessed. EEC guidelines for the toxicity testing of medicinal drugs define *ocular irritancy* as "the whole of reversible ocular changes of inflammatory source which appear after the application of a substance".

These studies must be performed when there is any possibility that the eyes may be voluntarily or accidentally exposed to the test substance, either because the substance is properly administered by this route or because there exists a risk that the substance will accidentally come into close proximity with the eyes. Sertaconazole gel, like the gel formulation of any other drug, falls into the latter category as its capillary or dermal application may result in an ocular insult.

Sertaconazole, a new antifungal agent, has been reported in both preclinical [1–3] and clinical studies [4–6] to have a wide spectrum of activity

against yeasts, dermatophytes and opportunistic filamentous fungi.

Material and methods

Health authorities are currently undecided on the definitive criterion that must be applied in studies evaluating the ocular tolerance of a chemical substance. Although the replacement of *in vivo* studies by one or more *in vitro* tests has not yet been validated, there already exists agreement on the fact that such tests can have a high predictive value [7].

For this reason, the tolerance of sertaconazole gel was evaluated by *in vitro* and *in vivo* methods. Although in a previous study sertaconazole was found to be excellently tolerated by skin [8] and mucous membranes, and although it is known that its pH is within the range acceptable for *in vivo* testing, the *in vitro* test was conducted first. The *in vitro* test selected was the measurement of the opacity and permeability of bovine cornea [9]. This is because the best approximation to the *in vivo* response is obtained in studies using isolated target organs [7] and, furthermore, since the corneae were obtained from cattle intended for human consumption, death of additional laboratory animals is avoided. In view of the results, the *in vivo* tolerance was confirmed by conducting the modified Draize test according to OECD Guideline No. 405 [10].

The substance to be tested was sertaconazole gel with composition identical to the commercial preparation Dermofix®. Ketoconazole gel (Panfungol®) was used as a reference substance.

Bovine corneal opacity and permeability test

This experiment was carried out in the Centro de Investigación y Desarrollo Aplicado, SAL, and was based on a modification of the method of Gautheron [9]. This method consists in exposing ox corneas to a volume of 0.75 ml of the test substance at 100% concentration for 10 min.

The ox eyes were obtained from animals which had been killed 2 h before testing and were supplied by the slaughterhouse of Sabadell (Spain).

The corneas were dissected from the eyes, leaving a 2–3 mm ring of sclera around them. They were then placed into special plastic chambers, which were subdivided internally into anterior and posterior compartments. The chambers were filled with Eagle's minimum essential medium (MEM) supplemented with 1% fetal calf serum and allowed to incubate at 32 °C for 1 h. Before exposure of the corneas to test substance, a baseline measurement of their opacity was obtained, and only those corneas with values between +3 and −3 were accepted. Once the corneas were chosen, they were exposed to test substances by removing MEM from the anterior compartment of the chamber, which was then filled with the test or control substances. Saline solution was used as a negative control and 2-ethoxyethanol as a positive control. After exposure to the corneal epithelium for 10 min, the chamber was washed by refilling it with MEM. Opacity was immediately read on an opacitometer, and a second reading was performed after 2 h.

Permeability was evaluated by applying 1 ml of 0.4% fluorescein solution in phosphate buffer to the epithelium of the cornea. After incubation for 90 min, the optical density of the medium in the posterior compartment of the chamber was read after verifying that the optical density of the fluorescein solution was within the set ranges.

Once the values of opacity and permeability had been determined, the *in vitro* corneal lesion index was calculated according to the following formula:

In vitro index = opacity + (15 × permeability)

Modified Draize test

This test was conducted in the Centro de Investigación Grupo Ferrer, Department of Toxicology, according to the methods described in OECD guidelines for testing of chemical products [10].

The experimental system used was female albino New Zealand rabbits, which were supplied by Pi Petit Farm (Barcelona). The animals were kept under controlled conditions that complied with EEC guidelines for housing and experimentation of animals for research purposes [11]. After the quarantine period, the animals were carefully examined to determine their health status and confirm the absence of ocular lesions that might adversely influence the evaluation. Rabbits were housed individually throughout the experiment.

Five animals per group were used. A single dose (0.1 ml) of test substance was applied to one of the eyes of each animal; the untreated eye was used as a control.

The treated eyes were not washed until 24 h after administration. The evaluation of the lesions was carried out 1, 24, 48 and 72 h after administration. If at the last evaluation no new lesions had occurred, the study was considered to be completed. Otherwise, the observation time was prolonged to 21 days. After examination at 24 h, one drop of sodium fluorescein ophthalmic solution was applied, this, after being removed with

another drop of NaCl solution, allowed improved observation of the corneal lesions, which remained temporarily yellow stained.

This method comprised the independent evaluation of three ocular structures—cornea, iris and conjunctiva—in addition to any other effects, such as chemosis. When the assessment scale for the irritant effect was scored, the minimum value indicating a positive aggression was 1 for cornea and iris and 2 for conjunctiva and chemosis.

Table 1. Opacity and permeability test

Experimental group	*In vitro* index
Negative control	
Mean	3.33
SD	2.55
Positive control	
Mean	62.37*
SD	14.95
Sertaconazole gel	
Mean	2.43
SD	1.80
Ketoconazole gel	
Mean	12.57*
SD	5.58

Mean index and standard deviations. * $P \leq 0.05$, Mann–Whitney's *U*-test, versus negative control. Sample size was $n = 3$ corneas per group.

Statistical analysis

Mann–Whitney's *U*-test was used to compare the results among the different experimental groups.

Results

The results obtained are shown in Tables 1 and 2. Data are expressed as mean values from each treatment group.

Discussion

As regards the *in vitro* results (Table 1), both substances are classified of minor irritant potency out

Table 2. Ocular tolerance/corrosion assay

Parameter	Time after exposure				
	1 h	24 h	48 h	72 h	21 days
Corneal opacity					
Sertaconazole gel					
Mean	0.600	0.200	0.000	0.000	–
SD	0.418	0.274	0.000	0.000	–
Ketoconazole gel					
Mean	2.000*†	2.000*†	1.600*†	1.600*†	0.200
SD	0.000	0.000	0.548	0.548	0.447
Iridal lesions					
Sertaconazole gel					
Mean	0.300	0.000	0.000	0.000	–
SD	0.274	0.000	0.000	0.000	–
Ketoconazole gel					
Mean	0.500†	1.000*†	0.800*†	0.900*†	0.000
SD	0.000	0.000	0.274	0.224	0.000
Conjunctival redness					
Sertaconazole gel					
Mean	0.000	0.100	0.000	0.000	–
SD	0.000	0.224	0.000	0.000	–
Ketoconazole gel					
Mean	2.000*†	2.800*†	2.000*†	2.000*†	0.000
SD	0.000	0.447	1.000	0.707	0.000
Chemosis					
Sertaconazole gel					
Mean	0.700†	0.200	0.000	0.000	–
SD	0.274	0.274	0.000	0.000	–
Ketoconazole gel					
Mean	1.800*†	2.200*†	1.800*†	2.000*†	0.000
SD	0.447	0.837	1.304	0.707	0.000

Mean scores and standard deviations. *$P \leq 0.05$, Mann-Whitney *U*-test, versus sertaconazole gel. †$P \geq 0.05$, Wilcoxon's test, versus control. Sample size was $n = 5$ animals per group.

of four possible categories, i.e. they are both considered to be slightly irritant. However, the very slight changes caused by sertaconazole gel are of the same magnitude or slightly less than those caused by the saline solution. Ketoconazole gel was 5.25 times more irritant than sertaconazole gel, the differences between its *in vitro* index and that of the control group being statistically significant. These results are particularly interesting because the cornea is one of the most important structures affected by ocular lesions induced by chemical substances.

These results corroborate those of the *in vivo* tests (Table 2), which showed that the irritant effect of sertaconazole gel never reached the minimum values considered to represent an injury in any of the structures studied. In contrast, the irritant effect of ketoconazole gel exceeded the minimum values in all cases at some examination time. According to the terminology used in this type of study, both gel formulations have an acceptable ocular tolerance. The difference between the group treated with ketoconazole and its corresponding controls was statistically significant, whereas that between animals treated with sertaconazole gel and controls was not. The differences between the groups treated with sertaconazole gel and ketoconazole gel are also significant.

This low irritant effect on one of the most sensitive epithelia confirms the excellent tolerance to sertaconazole gel shown by intact skin, scarified skin [8] and mucous membranes. This finding gives this type of topical treatment an important advantage in treatment of skin that is in a state of high sensitivity.

Both antifungal gels have a slight irritant effect according to the two methods of evaluation used, but remain within the accepted limits for this type of tolerance test. However, the tolerance of sertaconazole gel has always been higher than that of ketoconazole gel.

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