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Percutaneous Absorption of Indomethacin from Transparent Oil/Water Gels in Rabbits

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Abstract—The percutaneous absorption of indomethacin from transparent oil in water gels (TOW gels) has been studied in rabbits and compared with absorption from a hydrophilic gel and from a spray formulation. The area under the curve and the C_{max} values in plasma were significantly higher for the TOW gels in comparison with the other formulations after single application. The pH of the aqueous phase of the TOW gels did not significantly influence the bioavailability. After multiple application the TOW gels induced a larger increase in AUC (vs first application) in comparison with the other formulations. None of the formulations without drug damaged the skin after multiple application. For indomethacin formulations skin damage was more pronounced with the hydrophilic gel than for the TOW gels and spray formulation.

To overcome the disadvantages of indomethacin, several attempts have been made to develop a topical dosage form having local activity without systemic toxicity.

Gels are widely used as dermatological vehicles. Besides oleogels and hydrogels, transparent gels containing water, oil and one or more surfactants have been described and used extensively in cosmetics and pharmaceuticals. The terminology 'transparent oil-water (TOW) gels' was introduced by Provost; such gels were described as jelly-like transparent semi-solids which are optically isotropic and thermodynamically stable (Provost & Kinget 1988).

The present study was undertaken to evaluate the stability and the topical bioavailability of indomethacin from TOW gels prepared as described by Provost & Kinget (1988) and consisting of a quaternary system with two emulsifying agents, an oily liquid and water.

Materials and Methods

Composition and preparation of TOW gels

Materials used for the preparation of the gels were: two non-ionic surfactants, polyoxyethylene (30) cetostearyl alcohol (Eumulgin B3, Henkel, Dusseldorf, Germany) and polyoxyethylene (7) glycerol stearic acid ester (Cetiol HE, Henkel, Dusseldorf, Germany)), isopropylpalmitate (S.C. Federa, Brussels, Belgium) as the lipophilic component, and distilled water.

The composition of the different formulations is shown in Table 1. The TOW gels were prepared by heating a mixture of Cetiol HE, Eumulgin B3 and isopropylpalmitate in a water bath at 75°C. Indomethacin (Flandria Laboratoria, Zwijnaarde, Belgium) was solubilized in the melt. Water (75 C) was added and the preparation was stirred and allowed to cool to room temperature (20°C).

Stability study of indomethacin

The stability study was performed on formulation 2 (Table 1). The influence of the pH of the water phase on the stability of indomethacin was evaluated using four different buffer solutions (pH 6, 7, 8 and 9). The composition of the buffers was: 0·0593 M KH_2PO_4 and 0·0074 M Na_2HPO_4 (pH 6); 0·0275 M KH_2PO_4 and 0·0391 M Na_2HPO_4 (pH 7); 0·0025 M KH_2PO_4 and 0·0642 M Na_2HPO_4 (pH 8); 0·046 M $Na_2B_4O_7$ and 0·008 M HCl (pH 9). All ingredients were analytical grade.

The final pH of the TOW gels was measured and if necessary adjusted to the pH indicated above. The gels were stored for 50 days at 30°C.

Indomethacin assay

An HPLC method developed by Jonkman et al (1983) was modified and used for both stability determination and bioavailability experiments. The system included an HPLC pump (Merck-Hitachi, Model L-6000, Tokyo, Japan), a UV detector (Merck-Hitachi, Model L-4000 UV, Tokyo, Japan) set at 320 nm, an automatic integrating system (D 2000 Chromato-Integrator, Merck-Hitachi, Model 4000 UV, Tokyo, Japan, a Valco syringe CV-6-4 MPa-M60 injector (Valco Instr. Corp., Eke, Belgium) and a reversed phase column (Lichrospher 100 RP-18 (5 μm 125 × 4 mm), Merck, Darmstadt, Germany). The eluent solution consisted of tetrabutylammoniumphosphate (0·005 M) dissolved in methanol–water (65:35; v/v). pH was adjusted to 6·8 using 0·5 M NaOH. The operating flow rate was 1 mL min^{-1} and the temperature ambient.

Sample preparation for determination of stability

For each analysis a 1 g sample was weighed in a 50 mL beaker and dissolved in 20 mL methanol. The solution was quantitatively transferred into a 100 mL volumetric flask and the volume was made up to 100·0 mL with methanol. Five mL was transferred to a 50 mL volumetric flask and 5·00 mL of the internal standard solution (20 μg mL^{-1} niflumic acid in methanol) and 22·5 mL methanol were added. The volume was made up to 50·0 mL with water. A 20 μL sample was injected into the chromatograph. A calibration curve, prepared using known concentrations of indomethacin, was linear up to at least 20 μg mL^{-1} ($y = 4{\cdot}21x + 0{\cdot}11$; $r^2 = 0{\cdot}9999$).

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Table 1. Composition and appearance of TOW gels (indomethacin 1% w/w).

Formulation	Isopropyl palmitate	Eumulgin B3	Cetiol HE	Water	Appearance	Crystallization
1	2	15	20	62	liquid	–
2	5	15	20	59	semi-solid	–
3	10	15	20	54	semi-solid	–
4	5	10	20	64	semi-solid	–
5	5	20	20	54	semi-solid	+
6	5	18	10	66	semi-solid	–
7	5	18	25	51	liquid	–

Plasma assay

Plasma 250 μL, acetonitrile 200 μL and internal standard solution 50 μL were transferred to 5 mL glass, stoppered tubes. The mixture was vortexed for 120 s and centrifuged at 3000 rev min^{-1} for 10 min. The supernatant (250 μL) was transferred to a glass tube containing 1 mL citrate buffer (pH 3) and 2·5 mL of a chloroform–isopropanol mixture (9:1; v:v). The tubes were vortexed for 2 min and centrifuged at 3000 rev min^{-1} for 10 min. Two mL of the organic layer was transferred to a glass tube and dried at 45°C under nitrogen. The residue was redissolved in 200 μL of the mobile phase and 20 μL was injected into the chromatograph. The calibration curve, prepared using known indomethacin concentrations in plasma, was linear up to at least 40 μg mL^{-1} ($y = 4{\cdot}25x + 0{\cdot}19$; $r^2 = 0{\cdot}9991$). The within-day variation of the slope of the calibration curve was ±3·8%; the between day variation was ±4·0%.

In-vivo bioavailability study

Single application. Six male white rabbits were used for each experiment (Witte van Dendermonde, 3 kg ± 300 g). The animals were fasted for 24 h and their back skin was mechanically shaved 12 h before the experiment. Indomethacin was administered in one of five ways; as a solution in the marginal ear vein (Indocid amp. 25 mg mL^{-1}, MSD, Brussels, Belgium) or as a hydrophilic gel (Indocid Gel 1%, MSD, Belgium), a spray (Dolcidium spray 4·85%, SMB, Brussels, Belgium) or two TOW gel formulations (formula 2 with a pH of 4 and 7, respectively).

Formulations were applied on the rabbits' dorsal skin over a 7·5 × 7·5 cm area after removal of the animals from their boxes. During application, the rabbits were housed in individual boxes where only restricted movements were allowed for a 12 h period. The length of the boxes was chosen so as to avoid contact of the rabbit's back skin with the upper part of the boxes. This procedure was followed for single and multiple application experiments.

One mL blood samples were taken from the marginal ear vein 5 min before and every hour up to 12 h after dosing. Plasma samples were prepared and kept at 20°C before analysis.

Multiple application. Six male white rabbits (Witte van Dendermonde, 3 kg ± 300 g) were used for each experiment and the animals were kept and prepared as described above. Six different formulations were used for the experiments: the hydrophilic gel (Indocid, MSD), the spray (Dolcidium spray, SMB), the TOW gel (pH 7) and each formulation without indomethacin. Each formulation was applied once daily for 7 days (or 40 mg indomethacin per application). Blood samples were taken after the first and after the seventh applications from the marginal ear vein 1, 2, 3, 4, 6, 8 and 10 h after dosing. Plasma samples were treated as before. After the final blood sampling, skin biopsies were taken from the site of application. Biopsies were fixed in a mixture of ethanol 94°–formol–acetic acid (80:15:5; v/v), embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin. Evaluation was by light microscopy.

Statistical analysis of the pharmacokinetic data was performed by the Mann-Whitney U test and the two-tailed Wilcoxon-test ($P \leqslant 0{\cdot}05$).

Results and Discussion

Provost & Kinget (1988) defined a concentration range for the semi-solid formulations of drug-free TOW gels consisting of isopropylpalmitate, Eumulgin B3, Cetiol HE and water. In a first approach the influence of indomethacin on the consistency and appearance of the TOW gels was investigated (Table 1). Only formulation 6 seemed unable to solubilize the indomethacin, drug crystals being observed after preparation. Formulations 1 and 7 were clear liquids while formulations 3 and 5 were too viscous to be easily applied on the skin. Formulation 2 showed an acceptable semisolid consistency and was chosen for further experimental work.

Indomethacin is practically insoluble in water and, although soluble in alkaline solutions, it is unstable under these conditions. The time required for 10% degradation of the drug (t10) in a polysorbate 80 solution at room temperature was previously reported by Krasowska (1979); the shelf-life of indomethacin was dramatically increased by solubilization for pH values up to 9. At room temperature Krasowska reported t10 values of 45 days at pH 7, 9 days at pH 8, and 5 h at pH 9.

In our experiments no drug loss was observed after 50 days up to pH 7. Nine % drug was lost after 7 days at pH 8; and about 20% at pH 9. Tsai et al (1986) confirmed the increased stability of indomethacin using polysorbates in absorption ointments.

As reported for other micellar systems, the modifying effect on the rate of hydrolysis can be explained on the basis of the distribution of the drug between the bulk water phase and the micellar phase. The incorporation of the drug in non-ionic micellar systems protects it from attacking ions. Although there is no agreement on whether TOW gels should be considered as microemulsions, as macromicellar systems, stabilized emulsions or cubic liquid crystalline phases, the

Table 2. Bioavailability parameters (±s.d.) after a single topical application of 40 mg indomethacin with TOW gels, a hydrophilic gel and a spray, on rabbits (n=6). Statistical analysis of AUC and C_{max} was by the Mann-Whitney U test.

	TOW gel (pH 4)	TOW gel (pH 7)	Hydrogel	Spray
AUC ($\mu g\ mL^{-1}h$)	10·61±2·36	9·90±1·52	7·76±1·54*	8·19±2·15*
C_{max} ($\mu g\ mL^{-1}$)	1·45±0·33	1·31±0·38	1·12±0·14**	1·08±0·24**
t_{max} (h)	5	4	5	2

* $P < 0·05$ compared with TOW gel (pH 4) and with TOW gel (pH 7). ** $P < 0·05$ compared with TOW gel (pH 4) and with TOW gel (pH 7).

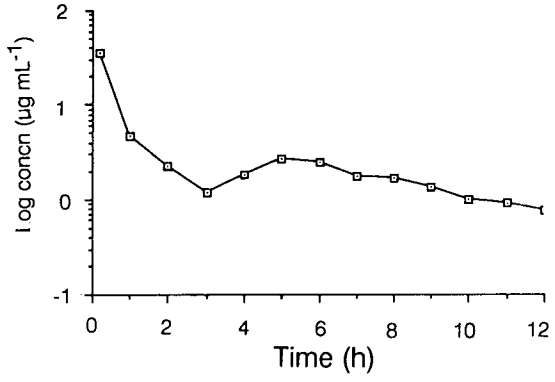

FIG. 1. Typical plasma concentration-time profile showing enterohepatic recirculation after i.v. administration of 40 mg indomethacin.

data reported suggest that TOW gels protect the drug from rapid degradation by alkali.

Provost et al (1989) compared the penetration rate of a hydrophilic and a lipophilic drug from TOW gels, as used here, through human skin in-vitro. They concluded that the penetration process was still the rate-limiting step and that the penetration rate for both drugs was comparable with formulations using other commonly used vehicles. As percutaneous resorption is a complex process resulting from several vehicle-drug-skin interactions, the absorption of indomethacin from TOW gels was studied after single and multiple applications and compared with other commonly used indomethacin formulations. Table 2 shows the $AUC_{0\ 10\ h}$, C_{max} and t_{max} values after a single application of 40 mg indomethacin formulated as a hydrophilic gel, as a topical spray and as two TOW gels (pH 4 and 7), on rabbits. Enterohepatic recirculation is a well known phenomenon for non-steroidal anti-inflammatory drugs. Duggan et al (1975) showed that enterohepatic recirculation of indomethacin is a species dependent phenomenon. In this study a distinct enterohepatic recirculation was seen after indomethacin was administered i.v. (Fig. 1). As the resorption proved to be a function of the apparent bile clearance and the gall bladder emptying rate, it seemed inappropriate to calculate absolute bioavailability data (Ritschel 1987).

Table 3. Bioavailability parameters (±s.d.) after a single topical application of 40 mg indomethacin and after the seventh application (once daily) with a TOW gel (pH 7), a hydrophilic gel and a spray on rabbits (n=6).

TOW gel (pH 7)	AUC ($\mu g\ mL^{-1}$ h)	C_{max} ($\mu g\ mL^{-1}$)	t_{max} (h)
Single appl.	8·91±1·26	1·56±0·19	3·6
After 7th appl.	18·64±1·85	2·93±0·54	3·2
Hydrophilic gel			
Single appl.	5·39±0·65	0·76±0·19	4
After 7th appl.	8·20±1·85	1·13±0·31	4
Spray			
Single appl.	7·40±2·57	1·08±0·24	2·5
After 7th appl.	12·10±2·92	1·80±0·59	2

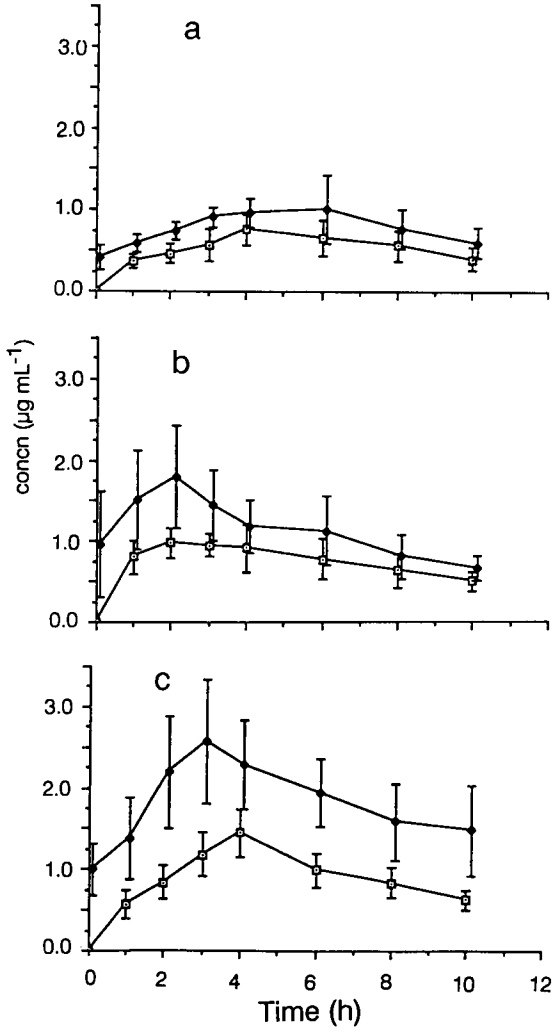

FIG. 2. Mean plasma concentration-time profiles after first (-□-) and seventh (-◆-) administration of dermatological formulations. (a) hydrogel, (b) spray and (c) TOW gel (pH 7).

The maximal plasma concentrations for both TOW gels were significantly higher in comparison with the hydrophilic gel and the topical spray (Table 3). For all formulations t_{max}

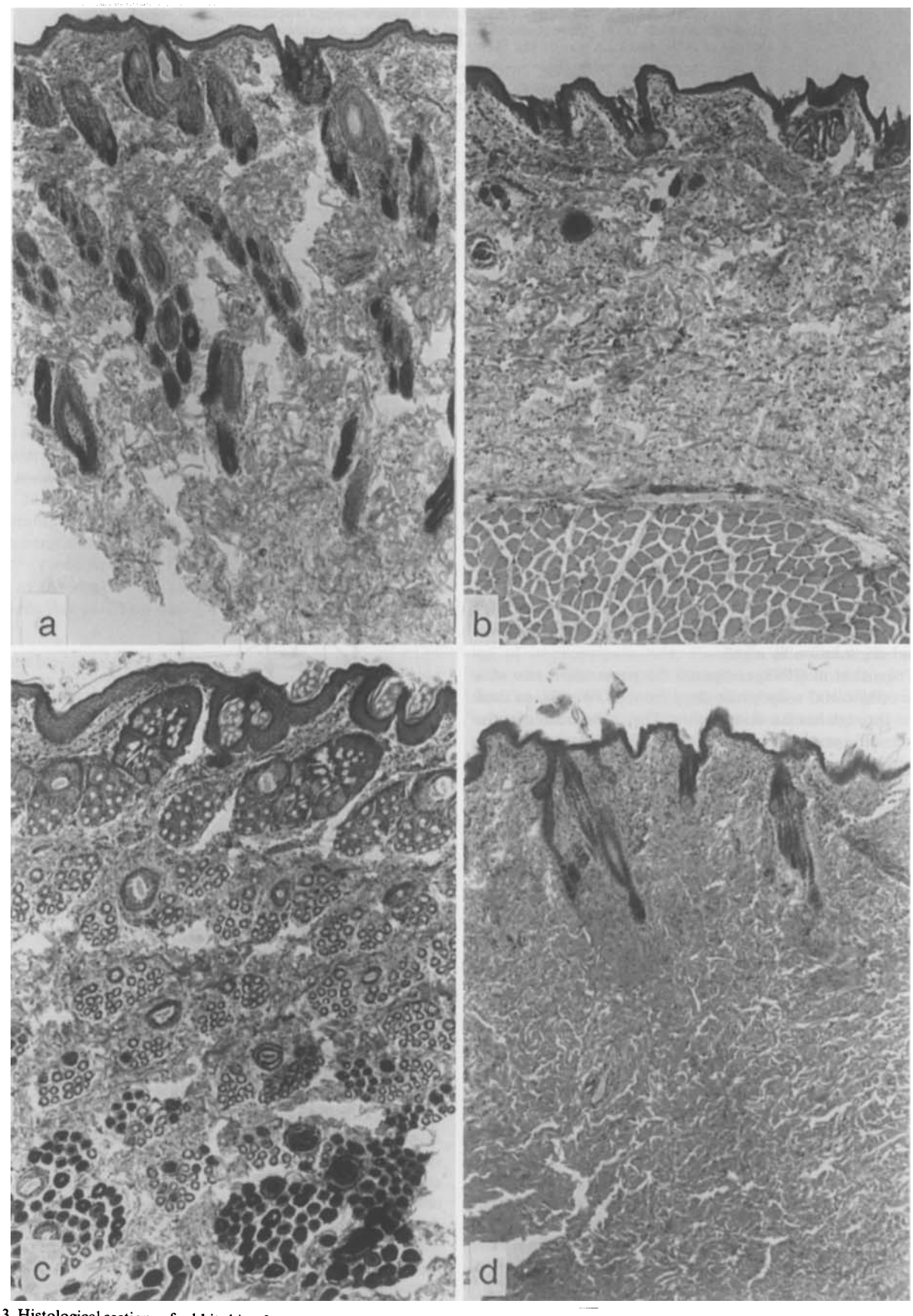

FIG. 3. Histological sections of rabbit skin after seven day treatment with (a) TOW gel without indomethacin (b) hydrogel (c) TOW gel and (d) spray formulation. (b), (c) and (d) are indomethacin-containing formulations.

values were around 4–5 h except for the Dolcidium spray where an average value of 2 h was noted. The $AUC_{0\text{-}10\ h}$ values were significantly higher for both TOW gels in comparison with the other formulations (Table 3). The relative bioavailability related to the hydrophilic gel was 131, 138 and 108% for the TOW gel (pH 7), TOW gel (pH 4) and the topical spray, respectively. The pH of the aqueous phase of the TOW gels did not influence the bioavailability significantly (Table 3). This was surprising as an influence of pH and degree of ionization on the percutaneous resorption of indomethacin has been reported (Tsai & Naito 1982).

Local therapy with indomethacin is usually by multiple application of the drug for several days. Fig. 2 shows the plasma concentration time profiles after the first and the seventh application (once daily) of a TOW gel (pH 7), the hydrophilic gel and the spray. At the time of the seventh application residual drug plasma concentrations were observed for all three formulations. Due to drug accumulation, a progressive increase in AUC and C_{max} was observed in comparison with the first application. However, the greatest increase in AUC was seen for the TOW gel. This could be due to the influence of non-ionic surfactants on skin permeability, resulting in a change in drug absorption rate and/or in the fraction of drug absorbed. Relative bioavailabilities after the seventh application were not calculated since steady state conditions were not reached.

Histological preparations from the biopsies of the different groups of formulations were examined. Histological sections of one control group and the groups treated with the indomethacin-containing preparations are shown in Fig. 3 (a-d).

The control slides (after 7 day application with the formulations without active substance) showed an undulating multilayered epidermis of moderate thickness. The epidermal appendages consisted of groups of 3 to 5 catagen hair follicles and small sebaceous glands that open at the surface in one follicular orifice. The dermis contained normal collagen bundles. In only one of the six slides was there a slight infiltration of neutrophilic polymorphonuclear cells and small amounts of lymphocytes around the capillaries of the upper dermis.

After the application of the hydrophilic gel containing indomethacin the epidermis appeared mostly thinner in comparison with samples from experiments with the control formulations. The horny layer was orthokeratotic with small foci of parakeratosis. In many places the epidermis was interrupted and small crusts of serum necrotic material and polymorphonuclear cell debris filled up the gaps.

Single or grouped polymorphonuclear leucocytes, lymphocytes and histiocytes were observed in the looser collagen of the upper dermis of all slides. The grouped hair follicles were in the end stage of the catagen phase and some were in the telogen phase. The mid and deep dermis were normal.

After application of TOW gels containing indomethacin the epidermis showed the same thickness as in the control slides where the TOW gel alone was applied. As in the preparations with hydrophilic gel containing indomethacin, there were some interruptions of the epidermis filled up with crusts of necrotic epidermal cells, serum and cell debris of polymorphonuclear cells. In all slides the capillaries of the upper dermis were dilated and surrounded by an infiltrate composed of polymorphonuclear cells, lymphocytes and histiocytes which also filled the dermal papillae. The grouped hair follicles were in the anagen phase. In two slides some early catagen hair follicles could be seen.

The fourth group of slides were from rabbit skin after 7 days' application of a spray formulation with indomethacin. The epidermis was very thin: the horny layer was orthokeratotic. The squamous cell layer was reduced to one or two flattened layers. The superficial dermis showed a subacute infiltrate composed of neutrophilic polymorphonuclear cells, lymphocytes and histiocytes. The larger part of the hair follicles was in early telogen and late catagen phases.

From these observations it may be concluded that the formulation without indomethacin caused no damage to the shaved rabbit skin after multiple applications.

The indomethacin-excipient combination is of importance. The most extensive damage to epidermis and upper dermis was observed with the hydrophilic gel, followed by the TOW gel. However, with this last preparation no atrophy of the epidermis occurred. Atrophy is most pronounced for the spray formulation where no necrotic crusts were found. The evaluation of the growth phase of the hair follicles requires comment. The different formulations were applied on different animals of the same 'age'. The histological aspects of the hair follicles were not examined before starting the treatment nor at sites adjacent to the treated areas. Therefore, data on this aspect of the study should be interpreted with caution.

In our opinion TOW gels may be considered as an interesting alternative for transdermal drug delivery. Further investigations on the influence of other TOW gel compositions within the same surfactant-oil phase composition, with other surfactant combinations and experiments with occlusive techniques will improve knowledge in this area.

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