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INVESTIGATION OF SOME TOPICAL FORMULATIONS CONTAINING DEXPANTHENOL

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Abstract: Owing to its ability to regenerate epidermal cells Dexpanthenol (D-panthenol; chemically known as (+)-2,4-dihydroxy-*N*-(3-hydroxypropyl)-3,3-dimethylbutyramide) has found use for the treatment of patholytic ileus and postoperative distention. The purpose of research was to develop a gel containing dexpanthenol by monitoring the effect of various concentrations of a gelating agent on the activity of the ciliary apparatus. A system containing 2.5% of hydroxyethylcellulose was optimal for the preparation of the gel. Together with a formulation containing 5% of dexpanthenol, drops with equal concentration of the active compound were tested for comparison. Physical characteristics, such as osmotic pressure, acidity, density and viscosity of the preparation were determined as well as its microbiological sterility. The anti-inflammatory activity of the gel was determined following its topical application. Epidermal tests showed its good tolerance after topical application to the shaved skin of guinea pigs.

Keywords: topical application; criteria of evaluation; dexpanthenol; anti-inflammatory activity; tolerance

Dexpanthenol, an alcoholic analog of pantothenic acid, has been known to be readily oxidized to pantothenic acid, a building block of coenzyme A (1-3). This, in turn, is indispensable for reconstruction of epithelial cells and is takes part in some biological cycles (4).

For the treatment of inflammatory conditions, it is essential to apply drugs not only affecting transmitters released during the inflammation, but also affecting cellular metabolism. In this respect, dexpanthenol, exerting anti-inflammatory and regenerative properties (5-9), is a candidate for topical treatment of inflammations. Moreover, owing to its hygroscopicity, it can control the humidity of both the epithelium and mucous membranes.

Dexpanthenol has been used extensively as a medicinal agent in various formulations. Among those applied topically are solutions, aerosols, ointments and creams.

In topical applications, an important role is played by the nature of an excipient in which the active compound is incorporated, which controls physicochemical characteristics of the entire formulation (10). In this respect, those hydrogel excipients are recommendable which ensure moisturizing, cooling and soothing properties, prolonged adhesion and good tolerance.

The primary purpose of this research was to develop an effective and safe hydrogel formulation with dexpanthenol for topical application to the skin and mucous membranes.

EXPERIMENTAL

Materials

Dexpanthenol (*Dexpanthenolum)* – Hoffman – La Roche Ltd., Basel, Switzerland; hydroxyethylcellulose (Natrosol *HR 250*) – Aqqualon, Duesseldorf, Germany; sorbitol – Ubichem Ltd., Steines, England. Methyl – and propyl 4-hydroxybenzoates – Synteza, Poznań, Poland; disodium phosphate and monopotassium phosphate – POCh, Gliwice, Poland.

Preparation of the hydrogel excipients

The excipients were prepared by addition of a weighed amount of hydroxyethylcellulose to a phosphate buffer of pH = 6.5 containing sorbitol and the preservatives. After 24 h, the homogenized excipient was autoclaved at 120°C for 20 minutes. In this way, 7 hydrogel systems containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5% of hydroxyethylcellulose was prepared.

Preparation of the hydrogel systems with dexpanthenol

These were prepared by addition of a 1:1 dexpanthenol solution in sterilized water and filtered through a 0.2 μm Synpor membrane filter, to the sterile excipient. After homogenization by stirring,

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the systems were transferred to sterile glass vessels. The final composition of the formulation prepared for further tests was as follows (in %): dexpanthenol 5.0, hydroxyethylcellulose 2.5, sorbitol 0.4, methylparaben 0.066, propylparaben 0.033, disodium phosphate 0.185, potassium dihydrogen phosphate 0.38 and distilled water 91.436.

Preparation of dexpanthenol drops

The drops were prepared by dissolving sorbitol and preservatives in water followed by sterilization of the solution at 120°C for 20 minutes. After cooling, dexpanthenol was added under sterile conditions, the mixture was stirred, the solution was filtered through the Synpore membrane filter and transferred to sterile glass vessels. The concentration of dexpanthenol in the drops was 5%.

Methods

Determination of the activity of ciliary apparatus

These investigations were carried out with frogs. After decapitation, the ciliated epithelium lining the pharyngeal-oesophagal cavity of the frog was exposed. On one-half of the exposed mucous membrane the system tested was applied by a pipet. The other half of the mucosa served as the control. In the anterior segment of both halves of the ciliated epithelium, small quantities of a powdered dyestuff were applied which owing to the motoring action of the cilia moved towards oesophagus. The effect of the hydrogel on the movement of the dyestuff was then compared with the control.

Osmotic pressure

This was measured by an automatic osmometer (Knauer, Berlin). 2.

Acidity (pH)

Hydrogen ion concentration was measured by means of a N5170 (Metra Tronik, Warszawa, Poland) pH meter.

Density

The density was determined pycnometrically at 20°C following the procedure of the Polish Pharmacopoeia V.

Viscosity

Viscosity of the gels was measured by means of a Hoeppler viscometer B3 (VEB Kombinat. Medizin und Labortechnik, Leipzig, Germany).

Sterility

The sterility tests were performed by using aseptic membrane filters, according to the Polish Pharmacopoeia V.

Table 1. Physical properties of the dexpanthenol preparations studied

Formulation	Osmotic pressure mOsm/L g/cm³	pH	Density mPa.s	Viscosity	Sterility
Hydrogel	321	6.42	1.0225	3806	absence of living microbes
Drops	306	6.54	1.0106	1.3	absence of living microbes

Table 2. Comparative evaluation of the inflammatory conditions of guinea pigs treated with dexpanthenol hydrogel and hydrogel alone

Group of animals	Preparation used	Degree of inflammation after				
		1 h	4 h	8 h	24 h	48 h
I	Dexpanthenol gel	0.58±0.46	1.48±0.58	1.65±0.53	0.80±0.25	0.50±0.10
II	Gel without dexpanthenol	0.58±0.46	1.89±0.61	2.20±0.32	1.80±0.12	0.80±0.10
III	Control sample	0.58±0.46	2.07±0.40	2.29±0.22	2.07±0.27	0.86±0.10

Data are expressed as mean ± SD of 12 animals in each group

Arbitrary degrees of inflammation: 3 – acute, distinctly edged erythema
2 – moderate, faintly edged erythema
1– mild erythema
0 – absence of erythema

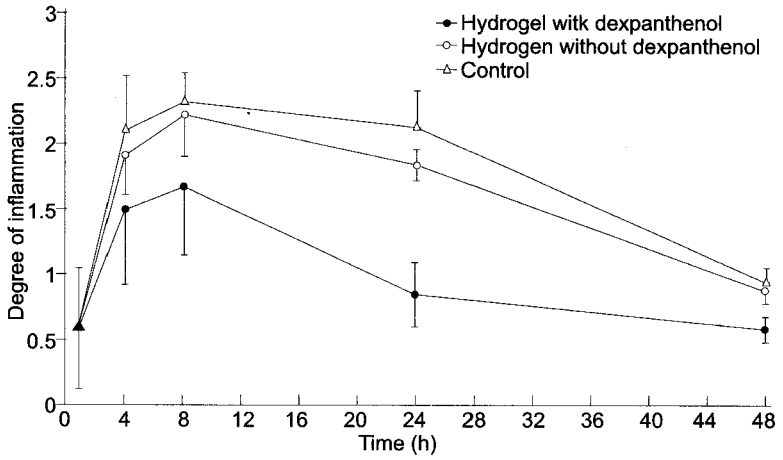

Figure 1. Temporal variations of the inflammatory reaction evoked by UV radiation in guinea pigs following application of hydrogel without and dexpanthenol in comparison with controls.

The results of the measurements are collected in Table 1.

Inhibition of inflammatory condition

These tests were conducted with red-eyed guinea pigs weighing *ca.* 250 g. A test of acute inflammation evoked by UV radiation was used. The animals were anesthetized by *i.p.* administration of Vetbutal and their backs were shaved. The shaved sites (three 2 cm x 2 cm areas) were then irradiated with a mercury lamp emitting the 240 nm UV radiation from the distance of 15 cm over 20 min. After one hour, on one of the irradiated sites 1 cm^3 of the dexpanthenol gel was laid, the other was covered with equal quantity of the gel without dexpanthenol and the third served as a control. The degree of inflammation was evaluated on a four-grade scale after 1, 4, 8, 24, and 48 h. The arbitrary degrees of inflammation adopted were: 3 – acute, distinctly edged erythema, 2 – moderate, faintly edged erythema, 1 – mild erythema, 0 – absence of erythema. At the time intervals indicated, successive portions of the gels were applied. The results are summarized in Table 2 and presented in Figure 1.

Estimation of the allergenic and toxic-irritating properties

This study was performed in a cohort of 23 hospitalized patients with allergic dermatoses and 7 healthy individuals, 13 women and 17 men aged between 18 and 67 yrs. The effects of the hydrogel and drops with dexpanthenol were evaluated by epidermal patch tests (11).

Statistical analysis

Statistical evaluation of the results was carried out using the Student's t-test. The level of significance was set at a = 0.05. Data are presented as mean ± SD.

RESULTS AND DISCUSSION

In an effort to develop a hydrogel with dexpanthenol for topical treatment, such criteria as isotonicity, isohydricity, density, viscosity, acidity, its interaction with the ciliary apparatus of mucous membranes and microbiological sterility have been applied. These parameters have been found to be responsible for good tolerance of the formulation applied to the skin and mucosa (12,13).

First of all, the effect of concentration of the gelating agent (hydroxyethylcellulose) in a formulation on the activity of the ciliary apparatus of the mucosa was investigated. The formulation to be developed should not impede the motion of cilia which play an important role in transportation. The hydrogel systems containing 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5% of hydroxyethylcellulose had different effects on the ciliary motion. Thus, at the hydroxyethylcellulose concentrations indicated, except for the 3.5% one, they did not affect the activity of the ciliary motion, while the latter concentration slowed down the motion. Also dexpanthenol drops and dexpanthenol gel had no ciliostatic effects. On this basis, the system containing 2.5% of hydroxyethylcellulose seemed to be optimal for the preparation of the dexpanthenol gel.

Secondly, the activity of the 5% depanthenol gel was compared with that of the drops of identical concentration of the active compound. Both formulations were prepared under aseptic conditions. The results of the measurements collected in Table 1 showed that the osmotic pressure of the preparations fell within the range of physiological standards. That of the body fluids is of the order of 300 mOsm/L. Apart from isotonic solutions, also weakly hypertonic ones are allowed which do not impede the ciliary activity. Moreover, suitable osmotic pressure of preparations is responsible for painlessness during application.

The acidity of the body fluids in a healthy individual, expressed in terms of the pH, ranges from 5.5 to 7.5. The preparations investigated here have pH close to those ensuring isohydricity. Again, the higher density and viscosity of the gel provide a prolonged contact time between the drug and the absorptive surface as compared to that of the drops. Both the density and viscosity of drug should not affect respective parameters of the mucosa in which cilia are moving. Further, microbiological assays revealed the absence of living microbes. In this respect both preparations meet the requirements set for drugs applied directly to wounds and extensive burns (Table 1).

Thirdly, the anti-inflammatory activity of the gel was tested on guinea pigs by using a UV test. According to the literature (14), the most reliable test for the determination of the efficiency of topical anti-inflammatory drugs is a test of acute inflammation. It consists in evoking inflammation by UV radiation followed by medical treatment of the exposed area. After a minor modification (extension of the irradiation time from 6 up to 48 h and application of the gel onto the shaved skin not before but after the UV irradiation to obtain a better insight into the anti-inflammatory efficiency) the test turned out to be useful for achieving goals of this study. Simultaneously, comparative tests were run with control group of guinea pigs and with a group treated with the excipient (Table 2 and Figure 1). Results of research have shown that the picture of inflammations evoked by UV irradiation in guinea pigs in the control group and those treated with the vehicle alone was similar. The differences were not statistically significant. Again, the hydrogel with dexpanthenol exhibited statistically significant inhibition of inflammation thus unambiguously indicating its anti-inflammatory efficacy. As seen, the highest efficiency of the preparation was noted between 8 and 24 h after irradiation.

The allergenic and toxic-irritating properties of the gel and drops with dexpanthenol were tested in the Dermatological Teaching Hospital of the Medical University of Gdańsk. Negative patch tests obtained with patients with allergic dermatoses and healthy individuals revealed the absence of any allergenic and irritating activity of the two preparations.

CONCLUSIONS

Considering all the studies, the tests have shown that both the composition of the preparation and the selection of the appropriate gelling agent at a specific concentration have led an optimal hydrogel formulation for topical treatment. The principal advantage of the formulation over drops is a better and longer contact of the drug at the site of application.

The modified UV test described in this paper seems to provide an adequate procedure for evaluation of topically applied anti-inflammatory drugs.

The determination of such parameters as the ciliary activity, osmotic pressure, acidity and viscosity provided useful criteria for evaluation of preparations applied not only to the skin but also to the mucous membranes of the nose, oral cavity and eye.

The results of this study clearly provide a sound basis for clinical trials for application of the dexpanthenol hydrogel for topical treatment of inflammatory conditions of the skin and mucous membranes.

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