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TECHNOLOGY AND PHYSICO–CHEMICAL EVALUATION OF SOLID OCULAR INSERTS CONTAINING SULFADICRAMIDE AND HYALURONIC ACID

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Abstract: Synthetic lipophilic membranes, containing sulfadicramide (SDC), disodium salt of hyaluronic acid and disodium salt of taurolithocholic acid were prepared as described (Fürst et al.). Rate constants of SDC release from the above membranes and its apparent permeability coefficients through the cornea isolated from the pig's eyes were determined.

Keywords: sulfadicramide, polymer ocular inserts, electrophysiologic study, drug release.

The reasons for relatively small bioavailability of the most ophthalmic drugs may be among others: the leakage of drug solution introduced into conjunctival sac, often irritating properties of an ocular drug or its excipients and also enhanced production of lacrimal fluid causing additional, uncontrolled elimination of the drug. Therefore, studies are conducted to obtain biocompatible formulations of ophtalmic drugs, characterised by high bioavailability in order to enable permanent control of the drug amount introduced into the eye and also to ensure high therapeutic comfort. These conditions are fulfilled in the case of underpalpebral therapeutic systems, which are the most often formulations that release the active substance following the zero–order kinetics and also in the case of inserts which are either soluble or insoluble in the lacrimal fluid and are characterised by the release following the first–order kinetics (1). At present, through adequate formulation it is possible to influence the kinetics and efficiency of the release of the active substance, enhancing at the same time its ability to penetrate the cornea and to reach the therapeutic concentration in the aqueous humour or vitreous body of the eye, if the penetration is therapeutically wanted (2).

Sulfadicramide (SDC) is a sulphonamide with properties similar to those of sulfamethazole. It is useful in the treatment of conjunctivitis and inflammation of the eye–lid. Ophthalmic drugs containing SDC are not produced in Poland.

However, SDC has been synthesized in the Department of Organic Chemistry, University of Medical Sciences in Poznań. Its molecular structure and physico–chemical properties were equivalent to Irgamid^R, which are produced by the Swiss Company Dispersa Ltd. (3).

The aim of the research reported in this paper was the physico–chemical studies of synthetic lipophilic membranes and the preparation of the inserts containing SDC with the addition of disodium salt of hyaluronic acid (HA) and sorption promoters. Isolated pig's corneas were used for biopharmaceutical studies of the above materials.

EXPERIMENTAL

Reagents

Sulfadicramide (Polfa, Poznań, Poland); disodium salt of hyaluronic acid (Sigma Aldrich Chemicals Co., Poland); glutathione oxidized (Roanal, Hungary); sodium salt of taurolithocholic acid (TLCH) (Sigma Aldrich Chemicals Co., Poland); all other reagents used were of analytical grade.

Apparatus

Fiske Osmometer (USA); Ussing chamber; Digital Electrometer V 530 (Meratronik, Poland); calomel electrodes K 401 (Radiometer Denmark); a source of constant electrical current; silver chloride electrodes; a perfusion apparatus for the determination of permeability rate of drugs (constructed in our laboratory); UV–VIS spectrophotometer Spekol (Carl Zeiss Technology, Germany); pH–meter (Cyberscan 500, Singapore); a shaking water bath, type 357 (Elpan, Lubawa, Poland).

Preparation of solid ocular inserts containing SDC

In order to obtain lipophilic membranes used further to formulate the inserts, on the bottom of a glass ring of 5 cm in diameter, 5 ml of the following solution was applied with the use of an automatic pipette: 2.0 g of lauryl alcohol and 100 g of a mixture consisting of ethyl alcohol, 99.8%; ethyl alcohol, 70% and ethyl ether, mixed at the weight ratio 1: 0.5: 8.5. Before mixing with other components, the required amount of disodium salt of hyaluronic acid (0.2 g) was dissolved in 70% ethanol. Then, 100 g of a mixture of collodion and ethyl ether, mixed in the ratio of 1:1, was added. After 24 hours of the membrane solidification, 5 ml of double distilled water was poured on the bottom of the rings, which was removed 30 min later and the membrane was dried and placed in a vacuum desiccator. Depending on the purity grade of the used collodion, two types of membranes were obtained: type A (reagent grade) and B (chemically pure). Physico–chemical evaluation of synthetic lipophilic membranes was performed according to previously described methods of the measurement of thickness, electric resistance [R] and conductivity [g] (4).

The prepared synthetic membranes were used for the formulation of bilayer inserts containing SDC. 50 mg of micronized SDC or a mixture containing SDC with a 1% addition of TLCH, was placed between two lipophilic membranes (method I and II).

Monolayer inserts were produced by the incorporation of the same dose of SDC (method III) or SDC and TLCH (method IV) into the structure of the lipophilic membrane. Because of a very high solubility of this drug in ethanol, SDC was dissolved in 70% ethanolic solution. Then the SDC solution was added to the remaining components of lipophilic membranes (ethyl ether and lauryl alcohol).

Measurements of electric resistance and conductivity of synthetic lipid membranes

The measurements were performed using membranes mounted in Ussing–type chambers, made from an organic glass, with an active surface area of approximately 1 cm^2. Between the chambers and the membrane silicon seals were mounted. They were prepared according to the protocol 184 Silicone Dow Corning to protect against marginal damage of the membrane and to ensure electric isolation between the compartments of the chamber. 10.0 ml of Hanks' electrolite solution was used to fill each of the Ussing's chamber compartments. To verify the appropriate preparation of the incubation solution the measurements of its osmolarity (300 ± 3 mOsm/l) were performed.

During the studies, measurements of voltage changes, ΔU, were performed. They were induced by the direct current pulses I with a density measured in μA per 1 cm^2 of the membrane area, which passed through the Ussing's chamber with mounted synthetic lipid membrane and filled with the incubation solution. The measured values were used for calculation of electric resistance R and conductivity g. The recorded change of voltage during the flow of constant current, was used to calculate the electric resistance R according to the equation:

$$R=U*I^{-1} \text{ where } V*A^{-1}*cm^2=\Omega*cm^2$$

The calculated values of resistance R were the sum of resistance of the studied lipid membrane and the resistance of incubation fluid filling the experimental chamber.

The measurements of conductivity were performed according to the protocol for electric resistance measurements using the equation:

$$g=I*U^{-1} \text{ where } A*V^{-1}*cm^{-2}=S*cm^{-2}$$

The obtained values of conductivity *g* were the difference between the conductivity of the incubation fluid and the values of conductivity obtained after mounting the membranes.

Conditions of kinetic studies

The rate of the SDC release from solid ocular inserts prepared in the four ways (I–IV), was measured at 37°C using a model perfussion apparatus described previously (5, 6). The inserts were mounted between the two compartments of the dialysis chamber. The receiving compartment was filled with 5 ml of Ringer's solution, pH=7.15 ± 0.05 and osmotic pressure 290 ± 10 mOsm/l.

0.1 ml samples were withdrawn from the receiving compartment at suitable time intervals until the system reached equilibrium state, and were immediately replaced with equal volumes of Ringer's solution. The absorbance of the samples was measured spectrophotometrically at the wavelength λ_{max}=258 nm against the Ringer's solution serving as a reference.

The permeability of SDC, released from the inserts through an isolated pig's cornea *in vitro,* was studied during four hours using a similar perfussion apparatus (5, 6). The Ringer's solution with a suitable addition of oxidized glutathione in the receiving

Table 1. Electrical resistance R and the conductivity g (x±SE) of the synthetic lipophilic membranes and Hanks' incubation solution (C) in the Ussing–type chamber at the probability p<0.0005.

Type of studied membrane	R [Ω * cm^2]	g [S * cm^{-2}]
Membrane A	177.57 ± 92.00 * 10^3 n=11	5.63 ± 1.05 * 10^{-6} n=11
Membrane B	62.63 ± 5.14 * 10^3 n=10	1.73 ± 0.11 * 10^{-5} n=10
C	89.61 ± 14.80 n=21	11.83 ± 1.90 * 10^{-3} n=21

Table 2. The rate constants of the release of SDC from the inserts and statistical evaluation of the results.

Method of the insert formulation	10^2 k [h^{-1}]	[r]	cv [%]	The yield of release of SDC [%]
I	7.888 ± 1.206	0.974	0.41	58.5
II	7.521 ± 1.470	0.963	0.61	68.1
III	7.853 ± 1.274	0.979	0.76	89.2
IV	9.084 ± 1.785	0.974	0.94	91.0

Where:
k – first–order rate constant with the confident interval at the probability $\alpha = 0.05$ for n = 13, 12, 10 and 10, respectively
r – correlation coefficient
cv – coefficient of variation

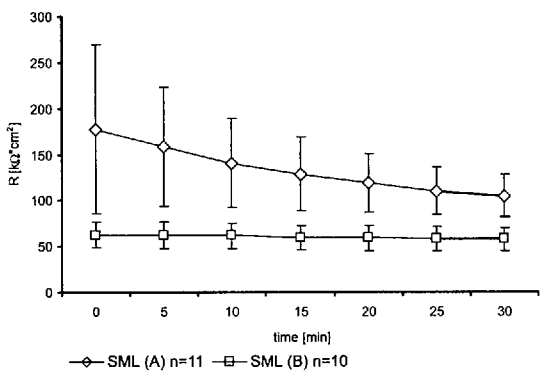

Figure 1. Electrical resistance R of the synthetic lipophilic membranes [SML(A) and SML(B)] during incubation in Hanks' solution.

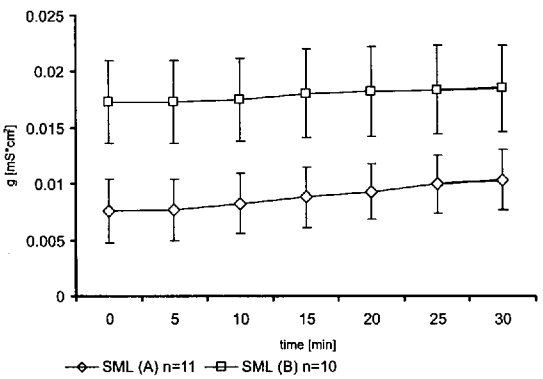

Figure 2. Conductivity *g* of the synthetic lipophilic membranes [SML (A) and SML (B)] during incubation in the Hanks' solution.

compartment, was equilibrated with the mixture of CO_2 and O_2 mixed at the ratio of 5% : 95%

RESULTS

The preliminary evaluation of the synthetic lipid membranes (series A and B), used in the studies, was based on the measurement of the thickness, which was equal to 20 ± 4 μm for both series. Physico–chemical analysis included the measurements of the chosen electric parameters: electric resistance R and the conductivity *g* of the membranes of series A and B and the incubation solution filling the Ussing's chamber (Table 1).

The values of the electric resistance measured during the experiments included the sum of resistance of the studied membranes and the incubation solution. During the analysis, the difference of electric resistance ΔR between the values of resistance on the membrane, mounted in the chamber and the resistance of the incubation fluid, filling the chamber, was measured. The calculated ΔR values show the actual resistance of the membranes (Figure 1).

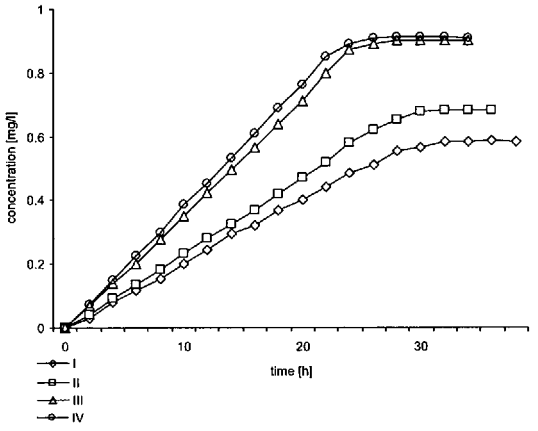

Figure 3. The concentration of SDC released from the solid ocular inserts obtained using methods I–IV as a function of time.

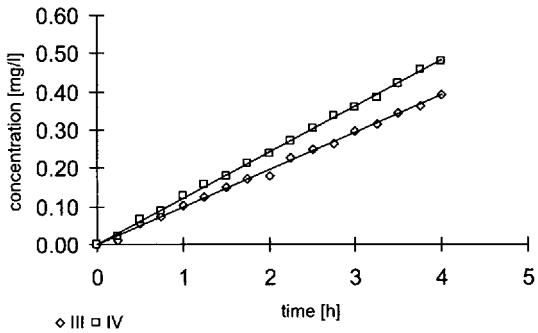

Figure 4. The changes of concentration of SDC released from the inserts, obtained using the methods III and IV, in the studies of rate of dialysis across the eye cornea *in vitro*, as a function of time.

Similar evaluation of changes of conductivity *g* was also performed for both series A and B lipid membranes mounted in the Ussing's chamber (Figure 2). The values do not describe the actual conductivity characterising a given membrane, but the degree of reduction of the value of the conductivity of the incubation fluid as a result of mounting the membranes in the chamber.

The measurements of the electric parameters of the lipophilic membranes containing HA were performed as described above. As the incorporation of HA significantly reduced the value of the electrical current in both series of the studied membranes, the mean values of resistance and conductivity were compared.

The physico–chemical evaluation of lipophilic membranes enabled to choose the type B membranes containing HA for further analysis. The changes of SDC concentration in the receiving compartment upon its release from the inserts were interpreted as a function of time according to the first–order equation (Figure 3).

On the basis of the obtained results, semi–logarithmic graphs of the concentration of SDC, released from the inserts, as a function of time were constructed. The rate constants of the drug release were calculated (Table 2).

As a result of biopharmaceutical evaluation conducted with the use of isolated pig's corneas it was demonstrated the inability of SDC to permeate across that barrier upon release from the inserts prepared according to methods I and II. However, such abilities for SDC release from monolayer inserts prepared according to methods III and IV were demonstrated (Figure 4).

The calculated values of permeability coefficients for methods III and IV are equal to: $Pc=4.094\ 10^{-6} \pm 1.404$ cm s^{-1} (r=0.998, cv=1.53%) and $Pc=5.043 \pm 1.985$ (r=0.995, cv=1.89%), respectively.

DISCUSSION

The ophthalmic diseases are treated mainly by the drugs for local use and the drugs for systematic use are only rarely administered. The main problems in ocular therapy are the necessity to administrate the drug in the form of e.g. drops or ointments in a small volume or quantity and also the removal of the drug as the result of enhanced production of lacrimal fluid and the increased blinking. Thus, a superior aim in the formulation of the new forms of ophthalmic drugs is, among others, enhancing of drug release period and the time of drug's contact with the cornea epithelium and also the improvement of the permeability across the cornea into the inner structures of the eyeball if such a permeability is therapeutically required. The studies are conducted on various forms releasing the active substance into the eye in the controlled manner (e.g. inserts, liposomes, colloidal carriers, gel systems, nanomolecules) (1).

Synthetic membranes, used further for the formulation of ocular inserts containing SDC, either soluble or insoluble in the lacrimal fluid, were prepared earlier from such polymers as polyvinyl alcohol (PVA) and polyvinylpyrolidon (PVP) or were prepared on the basis of the lipophilic membranes (7, 8). The important issue is the physico–chemical evaluation of the prepared polymer membranes, which will be used in further studies. The measurements of electric parameters performed earlier, demonstrated a very small permeability of the membranes, which results from their lipid nature. The characteristic features of the studied

membranes were the high values of electric resistance and low values of conductivity, which indicate their limited permeability for ions as can be seen in Table 1. Moreover, varied values of those parameters in the individual series of the obtained membranes argued to search for factors crucial for the different permeability of the membranes. In the preliminary studies, the influence of the degree of chemical purity of collodion, used in the membrane preparation technology on the values of measured parameters, was evaluated and statistically important differences between them were demonstrated. Type A membranes exhibited higher values of resistance R and lower values of conductivity g in comparison with the membranes type B at the equal thickness. The membranes type A also exhibited a wider range of the obtained values, which may result from the significantly higher values of measured electrical resistance values and thus a small degree of permeability for ions (Figure 1, 2). The membranes type B exhibited higher stability of the measured parameters obtained during the incubation in Hanks' solution. The obtained parameters R and g of the membranes type A and B are close to the electrical parameters characterising such barriers as the skin or the epithelium of cornea and are more different from the characteristics of the permeable epithelia (9–11).

It is known that there are integral proteins in every model of the biological membrane isolated from the organism. They create hydrophilic pores allowing for penetration of ions and by this way they induce lower values of the electric resistance together with higher electrical conductivity than in the synthetic lipid layers. Experimentally, the phenomenon of the enhanced permeability of such membranes for ions may be induced by the incorporation, for instance, of ionophores (12, 13).

The influence of HA on the functions of biological membranes, e.g. visceral mesothelium, was estimated earlier (14).

The mucoadhesive systems are especially useful in the technology of the drug formulations. They enhance adhesion to the eyeball, enhancing by this way the time of drug contact with the cornea epithelium (15). Water-soluble derivatives of hyaluronic acid, incorporated into the structure of a lipid membrane, are slowly washed out by lacrimal fluid; so they may be kept on the surface of the eyeball until the renewal of the mucin layer of lacrimal film. Besides that, HA is a physiological component of the vitreous body, aqueous fluid and eye cornea. Enhanced bioavailability and biocompatibility of the ophthalmic formulations already described, are explained as the resultant of their viscosity, bioadhesive properties and also the ability to form salts with the cationic therapeutic substances (16, 17).

HA incorporated into the structure of the membrane was assumed to be a factor enhancing the permeability of those membranes for SDC. On the basis of the performed electrical measurements statistically significant influence of HA on the permeability of membranes type A and B was proved. The obtained results suggest also that the smaller degree of purity of the collodion used in the technology of membrane preparation influences significantly the enhancement of the membrane permeability. Therefore, the type B membranes were used in the technology of the inserts.

Because of the relatively low (3–13.2%) efficiency of the release of SDC from the obtained earlier solid ocular inserts and, demonstrated in our earlier studies, inability of SDC to permeate across cornea together with HA, the addition of the sorption promoter TLCH was also postulated (18).

Model lipophilic membranes of thickness 20 ± 4 μm containing the addition of HA were used for the formulation of inserts, taking into consideration the four methods described above. Such membranes make it possible to prepare a research model for the evaluation of basic processes of therapeutic substances transport *in vitro*.

The changes of concentration of SDC released from the studied inserts were interpreted according to the kinetics of the first order processes. From the obtained, semi-logarithmic plots of the concentration of SDC as a function of time, the values of rate constants of the drug release from the inserts were calculated (Table 2). The calculated mean values were obtained from the three experiments conducted independently with the use of different inserts. These results demonstrate that the enhanced release of SDC from the solid ocular inserts was obtained for III and IV methods of their formulation (Table 2, Figure 3). At the same time, the results obtained for the methods I and II in that aspect, are more favourable than the earlier ones that concerned the inserts containing this sulphonamide and the addition of sodium taurocholate, sodium taurodeoxycholate and sodium glicocholate (18). In the present studies, the much lower promoter effect of TLCH in comparison with other formerly used derivatives of cholic acids or polyoxyethylene-8 lauryl ether (6,8) was also demonstrated.

Although the rate constants of the release of SDC from the inserts prepared using the methods III and IV differ significantly (probability $\alpha=0.05$), the

yields of SDC release are similar (Table 2). Therefore, it seems to us that the method of formulation and the presence of HA in the membrane facilitating the release of SDC has a crucial influence and the promoter influence of TLCH is less important. The present research confirmed also, described earlier, higher rate of drug release from the monolayer inserts in comparison with the bilayer ones, obtained on the basis of lipophilic membranes (8, 18).

The determined values of the permeability coefficients (Pc) of SDC, released from the membranes, prepared using methods III and IV, through the cornea of the pig's eye *in vitro*, allowed to conclude that this transport follows the zero–order kinetics (Figure 4), which is in accordance with our earlier observations (8). Taking into consideration their confidence intervals, the values of those coefficients are close each other. This fact is confirmed by the observed much lower influence of the used sorption promoter on the SDC transport across that barrier.

On the basis of performed experiments the ability of SDC, released from the inserts, prepared using the methods I and II to permeate across the cornea, was not proved. It is the result of much lower dynamics of that drug release, which is probably caused by the fact that the additional solvent penetration into the inner part of the insert is necessary. The solvent penetration might occur through the ionic channels, which are created as a result of incorporation of HA or through the structural pores of the membranes. Besides, the presence of HA in the structure of lipophilic inserts may influence their enhanced biocompatibility, which is very important because of the contact of that drug formulation with the surface of the eyeball.

The methods of the preparation of the lipophilic membranes with the addition of HA that we worked out and their use in the formulation of solid ocular inserts containing SDC, should contribute to further optimisation of the pharmaceutical and biological availability of the drug used in ophthalmology.

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