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EFFECTS OF LIQUID CRYSTAL SYSTEMS BASED ON CHOLESTEROL ESTERS ON SKIN PERMEABILITY

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The mechanisms of the influences of liquid crystal systems based on cholesterol esters on skin permeability were investigated. The effects of liquid crystal systems based on cholesterol esters on the transcutaneous penetration of phenazepam from transdermal therapeutic patches were studied in in vitro conditions. Changes in the fluidity of phospholipid liposomes and liposomes prepared from stratum corneum lipids were studied using fluorescence spectroscopy. Liquid crystal systems based on cholesterol were shown to be effective enhancers of skin permeability.

Keywords: cholesterol esters, liquid crystals, transdermal.

Studies of means of administering drugs are a priority in current pharmacology. One relatively new means, with great potential, is the transdermal route [1, 2]. There is particular interest in chemical compounds able to alter the properties of the skin barrier, increasing its permeability for drugs. Enhancers include many classes of chemical substances - saturated and unsaturated carboxylic acids [3, 4], terpenes [5 – 7], alcohols [8, 9], etc.

Apart from these compounds, potential enhancers of transcutaneous permeability include cholesterol and its esters. As natural components of all cell membranes [10], including epidermis cells [11], cholesterol and its esters not only act on physiological processes occurring in the skin [12 – 14], but also have direct influences on the liquid crystalline structure of lipid membranes and the intracellular matrix [12, 15]. The ability of cholesterol and its esters to form a variety of liquid crystal systems (LCS) makes it possible to prepare liquid crystals with specified phase transition temperatures. In turn, this opens up a wide range of potentials for creating systems with characteristics close to those of liquid crystal epidermal structures and with efficient and direct influences on them at the temperature of the human body.

We studied the effects of LCS based on cholesterol esters on skin permeability for phenazepam and their effects on the structure of the stratum corneum and liposome fluidity.

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EXPERIMENTAL SECTION

The active compounds used in in vivo experiments were 7-bromo-5-(2′-chloro)phenyl-1,2-dihydro-3H-1,4-benzdiazepin-2-one (phenazepam) and 7-bromo-3-hydroxy-5-(2′-chloro)phenyl-1,2-dihydro-3H-1,4-benzdiazepin-2-one (3-hydroxyphenazepam), synthesized at the Physicochemical Science Research Institute, Ukrainian National Academy of Sciences.

The compositions of LCS based on cholesterol esters and fatty acids are shown in Table 1. All cholesterol esters were obtained from Sigma-Aldrich.

Skin permeability was studied in vivo using white mongrel male mice weighing 18 – 22 g and young male Wistar rats weighing 180 – 200 g, kept in standard animal-house conditions with free access to food and water.

Studies of skin permeability in mice were performed using transdermal therapeutic systems (TTS) prepared as follows: 50 mg of cholesterol ester LCS was melted, and phenazepam was added to the resulting melt and mixed thoroughly. The resulting TTS was poured into a mold and left to set to the solid state. The 1,4-benzdiazepine derivative concentration in the prepared system was 0.1 mg/cm^2. Skin permeability was studied in rats using TTS weighing 1.25 g, of area 25 cm^2. The phenazepam and 3-hydroxyphenazepam concentrations were 0.4 mg/cm^2. These TTS were of the matrix type.

The reference TTS consisted of water, polyvinyl alcohol (reagent grade), glycerol (reagent grade), polyethylene oxide

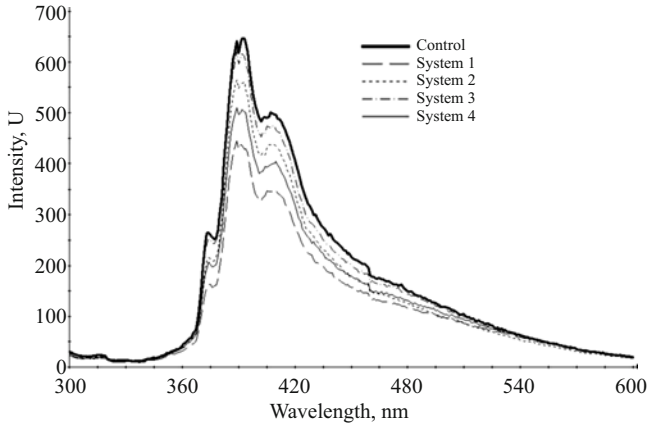

Fig. 1. Fluorescence spectra of pyrene in phospholipid membranes on addition of different LCS based on cholesterol esters at t = 37°C.

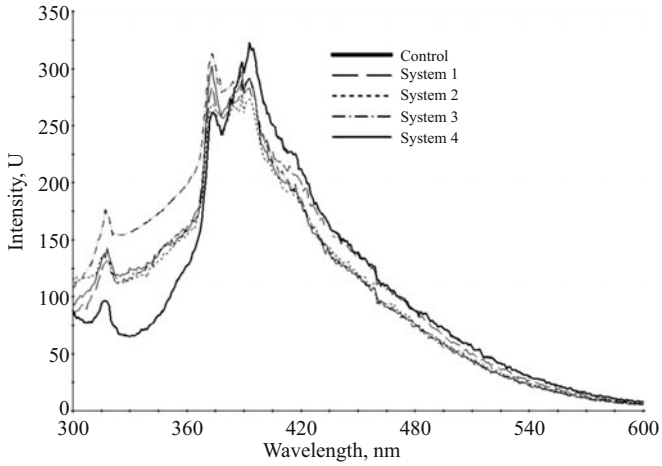

Fig. 2. Fluorescence spectra of pyrene in membranes based on stratum corneum lipids on addition of different LCS based on cholesterol esters at t = 37°C.

400 (reagent grade), and 1,2-propylene glycol (reagent grade), at a ratio of 4:2:1:1:2. The phenazepam concentration in the prepared system was 0.1 mg/cm^2.

TTS were applied to previously shaved skin in the interscapular area. Application time in mice was 2 h with an applied TTS area of 1 cm^2; in rats, application time was 6 h and applied TTS area was 25 cm^2.

Mouse experiments involved assessment of the quantity of penetrating substance in terms of the pharmacological response of the body via assessment of the minimum effective doses (MED) of corasol inducing clonic-tonic convulsions (CTC) and tonic extension (TE) when given into the tail vein of the experimental animals. The anticonvulsive activities of formulations were assessed in terms of the mean minimum CTC-inducing effective corasol dose (DCTC) and the mean minimum effective TE-inducing corasol dose (DTE). Controls consisted of DCTC and DTE values from animals not given anticonvulsant. Basal TTS consisted of reference TTS (composition as above). Corasol was obtained from Sigma-Aldrich.

In rat studies, the quantity of substance penetrating into the body was assessed by HPLC on an Agilent 1200 SD LC System chromatograph with a UV detector, using a stainless steel column of size 0.15 m by 4.6 mm filled with Zorbex Eclipse XDR-C_{18} sorbent with a particle size of 5 μm.

Effects on the physicochemical properties of membranes (fluidity, polarity) were assessed using membrane probes. The membrane probe used here was pyrene (Merck Chemicals), which was incorporated into phospholipid liposomes prepared from skin stratum corneum lipids. Pyrene fluorescence intensity was measured on a Varian Carry Eclipse fluorimeter. The temperatures at which fluorescence intensity was measured coincided with the phase transition temperatures for each of the systems studied. Liposomes were prepared as follows: pyrene, the permeability enhancer (LCS), and lecithin or skin corneum stratum lipids at a molar ratio of 1:10:100 were dissolved in chloroform, after which the chloroform was evaporated. The dry residue was taken up in water and shaken intensely for 10 min to form an emulsion. The resulting emulsions were sonicated for 10 min at a frequency of 22 kHz. The liposome concentration in the study solutions was 0.8 g/liter and liposome size ranged from 260 to 17,500 nm [16].

Skin stratum corneum lipids were obtained from skin stratum corneum prepared using the classical Bligh and Dyer method [17]. The stratum corneum was included in a chloroform:methanol:water (1:2:0.8) system. The extract was then diluted with one volume of water and chloroform. The lower chloroform layer was separated from the resulting biphasic system and evaporated in a rotary evaporator; the residue was dissolved in anhydrous carbon tetrachloride. The composition of the resulting lipid mix was: ceramides (50%), cholesterol (25%), fatty acids (10%), and neutral fats (15%).

Statistical processing was performed in Microsoft Office Excel 2003 and significant differences were identified using Student's test.

RESULTS AND DISCUSSION

Changes in the properties of biological membranes are often studied using fluorescent membrane probes. The membrane probe used here was pyrene, which can be used in both the free and bound states [18 – 20]. This probe was selected because changes in the ratios of the fluorescence intensity peaks of the pyrene molecule can reflect changes in fluidity and the polarity of the lipid bilayer [21]. Pyrene does not contain any polar groups and is incorporated into the bilayer at the level of acyl residues and allows changes in the internal part of the bilayer to be assessed. Data on changes in pyrene fluorescence intensity are shown in Fig. 1.

Changes in membrane microviscosity were identified using the ratio of the intensities of the fluorescence peaks at 470 and 370 nm (I_{470}/I_{370}), which corresponds to its excimer

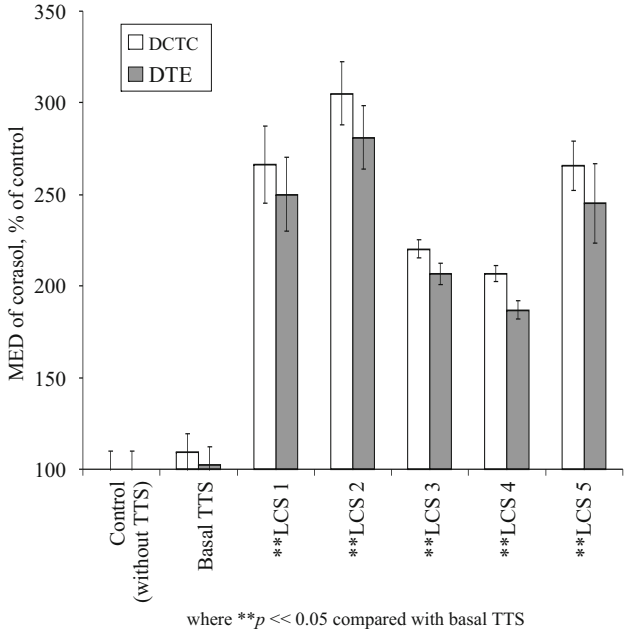

Fig. 3. Anticonvulsive activity of phenazepam given transdermally (dose 0.1 mg/cm^2).

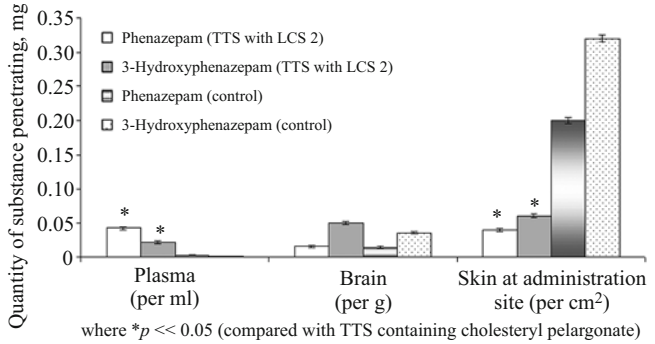

Fig. 4. Quantities of transdermal phenazepam and 3-hydroxyphenazepam entering the body (dose 0.4 mg/cm^2).

and monomer forms. These spectra show that the peaks corresponding to the excimer form were not seen. This led to the conclusion that liquid crystal systems based on cholesterol esters had no effect on the microviscosity of lecithin liposomes. We can suggest that in contrast to cholesterol, its esters do not occupy such a rigid position in lipid membranes and thus do not restrict the mobility of acyl residues of phospholipids. At the same time, there was a decrease in the coefficient of polarity (ratio of the intensities of fluorescence peaks at 370 and 390 nm (I_{370}/I_{390})) from 0.38 to 0.33. Decreases in the coefficient of polarity were seen on addition of all liquid crystal systems studied to liposomes. The most marked action on increases in hydrophobicity in the integral zone of membranes was seen with liquid crystal system 1, containing more than 50% short-chain esters.

Skin stratum corneum contains minor quantities of phospholipids, so data on the effects of LCS based on cholesterol esters on skin lipids were obtained using liposomes prepared from stratum corneum lipids. Stratum corneum lipids contained the following components: ceramides (~60%), cholesterol and its esters (~25%), and fatty acids (~15%). Data on the effects of LCS based on cholesterol esters on pyrene fluorescence intensity in liposomes made from stratum corneum lipids are presented in Fig. 2.

Addition of cholesterol LCS to liposomes made from stratum corneum lipids increased the coefficient of polarity of pyrene, I_{370}/I_{390}, from 0.77 to 1.07 when LCS 2 was used. This increase in polarity may be evidence for an increase in the inclusion of water in the composition of the lipid bilayer. Addition of all other systems also produced increases in the coefficient of polarity, though not to the extent seen with LCS 2. It should be noted that these assessments were supported by our previous studies [22].

Studies of the effects of LCS based on cholesterol esters on skin permeability for phenazepam in in vivo conditions were performed by determining the minimum effective doses (MED) of corasol inducing CTC and TE in experimental animals. The resulting data are presented in Table 3.

All the cholesterol ester-based LCS studied here had marked effects on changes in the MED of corasol as compared with control values. The greatest increases in DTE and DCTC were seen using LCS 2 as enhancer (DCTC 305%, DTE 281%). This high activity of LCS 2 can be explained in terms of the phase transition temperature, which corresponded to the temperature of the skin area to which the TTS was applied. High values were also seen for LCS 1 (DCTC 266%, DTE 250%) and LCS 5 (DCTC 265%, DTE 245%). The phase transition temperatures of these systems were also close to the skin surface temperature in the experimental animals, which was 28 ± 0.5°C (measurements were made using a thermocouple). The phase transition temperatures (Tale 1) of LCS 3 and 4 were notably greater than skin tempera-

TABLE 1. Composition of Liquid Crystal Systems Based on Cholesterol Esters

System	Composition	Phase transition temperature, °C
LCS 1	Cholesteryl pelargonate (45%), $t_{melting}$ = 90.8°C, cholesteryl valerate (25%), $t_{melting}$ = 93°C, cholesteryl succinate (30%), $t_{melting}$ = 179°C	27.5 – 36.5
LCS 2	Cholesteryl pelargonate (50%), $t_{melting}$ = 90.8°C, cholesteryl valerate (25%), $t_{melting}$ = 93°C, cholesteryl adipinate (25%), $t_{melting}$ = 195°C	24.5 – 31.5
LCS 3	Cholesteryl pelargonate (52.9%), $t_{melting}$ = 90.8°C, cholesteryl valerate (21.4%), $t_{melting}$ = 93°C	32.5 – 42.5
LCS 4	Cholesteryl pelargonate (85%), $t_{melting}$ = 90.8°C, cholesteryl propionate (15%), $t_{melting}$ = 95.2°C	37 – 45

ture. Thus, increases in DCTC and DTE using these systems were only slightly different from values for control TTS without permeability enhancer. Thus, our in vivo experiments supported the view that phase transitions influence changes in skin permeability.

HPLC was used to measure the absolute quantities of phenazepam and 3-hydroxyphenazepam penetrating from transdermal therapeutic systems based on LCS 2 (25 cm^2) in the transcutaneous route of administration in rats (Fig. 4). The reference control system consisted of TTS in which the permeability enhancer was cholesteryl pelargonate.

The greatest quantity of phenazepam was seen in plasma (0.043 mg/ml), while the greatest quantity of 3-hydroxyphenazepam was seen in skin (0.06 mg/cm^2). Calculations showed [23] that TTS based on LCS 2 led to absorption by rats of 60% of phenazepam and 69% of 3-hydroxyphenazepam, in terms of initial contents in TTS, across a skin area of 25 cm^2 and with an application period of 6 h. These values provide evidence of the high efficacy of LCS 2 as an enhancer of transdermal permeability.

Thermotropic liquid crystal systems based on cholesterol esters were effective enhancers of skin permeability when their phase transition temperatures coincided with the application site skin temperature.

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