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ON THE POSSIBILITY OF THERAPEUTIC ACTION AFTER TRANSDERMAL PATCH APPLICATION

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Background. As scientific knowledge about the peculiarities of the structure and functional properties of the skin increased, it became clearer that during transdermal administration, drug may accumulate in the deep layers of the dermis and subsequently get diffused into the bloodstream even after the transdermal therapeutic system (TTS), also called transdermal patch, had been removed. **Objective:** to quantify active drug substances remaining in an animal skin after TTS application. **Materials and methods.** Two previously developed transdermal patches containing Russian-made drug substances were chosen for the study: aminodihydrophthalazinedione sodium (immunomodulator) and bis(1-vinylimidazole-N) zinc diacetate (antidote for carbon monoxide). The study was performed on male Chinchilla rabbits weighing 2.5–3 kg. Five series of experiments were performed for each substance: immediately after removal of the patch, 4 hours later, at week 1, 2 and 3 after removal. High-performance liquid chromatography and atomic absorption spectroscopy methods were used to quantify residual drug substances left in the skin. **Results.** In the skin flap that was in contact with the aminodihydrophthalazinedione sodium TTS for 24 hours, 0.516 mg of the drug was detected immediately after removal of the patch. Over the next two weeks, the drug substance in the skin decreased with the immunomodulator significantly reducing to 0.41 mg in the first 4 hours. In the skin flap that had been in contact with zinc bis(1-vinylimidazole-N) diacetate for 24 hours, about 1 mg of the drug was present immediately after patch removal. Four hours after removal of the transdermal patch, the quantity of active substance in the skin remained practically unchanged. At week 1 and 2, the quantity of the antidote decreased slightly to ~0.7 mg and ~0.25 mg, respectively. **Conclusion.** For transdermal application of aminodihydrophthalazinedione sodium, the skin can act as a drug depot and prolong the effect of this drug even after the transdermal patch had been removed. No such effect was found in the case of bis(1-vinylimidazole-N) zinc diacetate, which is apparently due to the different solubility of the drugs in the biotissue.

Keywords: transdermal therapeutic system, residual drug, skin.

INTRODUCTION

One of the advantages of transdermal therapeutic systems (TTS) over traditional methods of drug administration is the immediate termination of the drug after removing the TTS from the patient's skin, thus preventing the development of several side effects of the drug and avoiding drug overdose [1, 2].

However, as scientific knowledge about the peculiarities of the structure and functional properties of the skin increased, it became clearer that during percutaneous administration, drugs can accumulate in the deep layers of the dermis and subsequently diffuse into the bloodstream even after the patch had been removed. For example, fentanyl, as a lipophilic drug, continues to be absorbed into the subcutaneous fatty tissue and remains in it almost 24 hours after removal of the patch from the patient's skin [3]. We did not find any other studies on the effects of TTS in the open press. It should be noted that the data obtained by studying the quantity of residual drug substance in the skin after removal of the transdermal patch can make significant changes in the TTS regimen.

The purpose of this work was to quantify active drug substances remaining in an animal skin after TTS application.

MATERIALS AND METHODS

The following two previously developed TTSs [4, 5] containing Russian-made drug substances were chosen for the study of the quantity of drug substances in the skin after removal of the transdermal patch:

1. Aminodihydrophthalazinedione sodium (trade name Galavit, SELVIM LLC). Molecular mass is 206 Da.
2. Bis(1-vinylimidazole-N) zinc diacetate (trade name Acizol, Favorsky Irkutsk Institute of Chemistry). Molecular weight 372 Da.

Since both drugs are hydrophilic, they were introduced into the patch as part of water-in-oil emulsion compositions. This approach is related to the fact that the

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intercellular space of the epidermal barrier, represented by a complex lipid mixture, is the main route of percutaneous penetration for most compounds [6].

Auxiliary substances and materials approved for medical use were used in the manufacture of TTS laboratory samples.

Microemulsion compositions with drug substances included the following components: purified water (FS 42-2620-97), 0.9% sodium chloride solution (Escom, Russia), sodium dodecyl sulfate (AppliChem Panreac, Spain), apricot kernel oil (Desert Whale Jojoba Company Ltd, USA), alpha-tocopheryl acetate (BASF SE, Germany), docusate sodium (Sigma, USA), and emulsifier Decaglyn PR-20 (Nikko Chemicals Co., Ltd., Japan). Foam tape 9773 (3M, USA), sorbent base PALV-01 (Palma Group of Companies LLC, Russia), and Scotchpak 9730 film (3M, USA) were used to create the transdermal patch.

The following reagents were also used: ethylenediaminetetraacetic acid (EDTA) (Sigma, USA), acetylcysteine (Sigma, USA), papain (Sigma, USA), government standard sample of aqueous solution of zinc ions (GSO 7837-2000), syringe filters (Agilent, cellulose acetate 0.45 μm, 25 mm).

Equipment used in the work: Dispergator (Heidolph DIAX 900, Germany); ultrasonic homogenizer (Heilscher UIS250V, Germany); analytical scales (GH-200 AND, Japan); centrifuge (Hettich Rotina 38R, Germany); liquid chromatograph (Agilent 1200, USA) equipped with a UV detector, autosampler, degasser and column thermostat; hotplate magnetic stirrer (IKA RT10, Germany); atomic absorption analyzer (Analyst A100, Perkin Elmer).

Research methodology

The study was conducted on male Chinchilla rabbits weighing 2.5–3 kg.

The animals were obtained from the breeding nursery of Krolinfo LLC. The producer provided a veterinary certificate of the last health check. All experimental animals were specially bred and had not previously participated in studies. They were quarantined for 14 days. All manipulations with the animals were performed according to the rules adopted in the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986).

Laboratory samples of the patch were attached on pre-shaved areas of the skin of the rabbit back at the base of the neck.

Five series of experiments were performed for each drug substance: investigation of the drug content in the skin immediately after removal of the transdermal patch, four hours, one, two, and three weeks after removal of the patch. After the rabbits were removed from the experiment using Zoletil 100 (Virbus Sante Animale, France) and Rometar (Bioveta, Czech Republic), a skin flap was taken from the place of the back where the patch was applied.

Methodology for quantifying the residual drug substances in the skin

The subcutaneous fat area (SFA) was separated from the dermis, and everything was crushed. Dissolution of the skin and subcutaneous fat was performed separately at 600 °C and under constant stirring in a 0.2 M phosphate buffer solution with the addition of EDTA, acetylcysteine, and papain.

The quantity of bis(1-vinylimidazole-N) zinc diacetate in the solution was determined by atomic absorption spectroscopy. Since the acyzol molecule contains zinc ion ($C_{14}H_{18}N_4O_4Zn$), we used the state standard reference sample GSO 7837-2000 of aqueous solution of zinc ions to construct the calibration curve. The conversion coefficient of zinc concentration to drug concentration was 5.7.

The quantity of aminodihydrophthalazinedione sodium in the solution was determined using the high-performance liquid chromatography technique we developed earlier [7].

A 600 μL skin solution was added to a 2.0 mL centrifuge microtube, 200 μL of 50% aqueous trifluoroacetic acid solution (by volume) was added. The mixture was stirred for 2 min and centrifuged at 6,000 rpm for 10 min. 500 μL of supernatant was transferred to a 1.5 mL microvial, 55 μL of 50% potassium hydroxide solution (by weight) was added, and the mixture was stirred.

Chromatographic determination was performed under the following conditions: chromatographic column: Mediterranea Sea 18 25 × 0.46 cm, 5 μm (Teknokroma Analitica SA, Spain) with an 8 × 4 mm pre-column filled with the same sorbent. Mobile phase: acetonitrile – 0.015% aqueous solution (by volume) of trifluoroacetic acid, pH = 2.5 (15 : 85). The mobile phase was prefiltered and degassed in a vacuum filtration device. Rate of flow of the mobile phase: 0.8 mL/min. Elution mode: isocratic. Column thermostat temperature: 25 °C. Sample volume injected: 10 μL. Detection wavelength: 221 nm. Retention time: Approx. 11.7 min. Chromatographic time: 16 min. Lower limit of aminodihydrophthalazinedione sodium quantification: 50 ng/ml. Linearity range of the technique: 50–2000 ng/mL.

Quantification of bis(1-vinylimidazole-N) zinc diacetate left in the patch after use

After removal of the transdermal patch, the TTS samples were cut into several pieces, placed in a 250 mL conical flask, and filled with 150 mL of distilled water. Extraction of drug substances was done from the TTS was done in a boiling water bath for 1 hour. This extraction was repeated 2 more times. Then the solution was

filtered through a paper filter into a 1000 mL volumetric flask and brought to the mark with distilled water. Then the obtained solution was diluted in a 1 : 25 ratio. The quantity of bis(1-vinylimidazole-N) zinc diacetate was determined in the solutions by spectrophotometric method at 225 ± 2 nm maximum absorption spectrum, using the following formula:

$$x = \frac{D_x \times m \times 25}{D_0},$$

where D_x is optical density of the test solution, D_0 is optical density of the control sample, m (in grams) is the mass of bis(1-vinylimidazole-N) zinc diacetate taken to prepare the control sample, 25 is the dilution factor.

To prepare a control sample, 0.015 g of bis(1-vinylimidazole-N) zinc diacetate was placed in a 1000 mL volumetric flask and diluted with distilled water to the mark.

Quantification of aminodihydrophthalazinedione sodium left in the patch after use

After removal of the transdermal patch, the TTS samples were cut into several pieces and placed in a 250 mL conical flask. They were filled with 0.5% alcohol-aqueous (1 : 1) sodium dodecyl sulfate solution (150 mL). The drug substance was released into the solution at 600 °C and constantly stirred on a hotplate magnetic stirrer for 1 hour and 45 minutes. Then the solution was filtered through a paper filter into a 500 mL volumetric flask. This extraction was repeated 1 more time. Then the volume in the flask was brought to the mark with 0.5% alcohol-aqueous (1 : 1) sodium dodecyl sulfate solution. The quantity of aminodihydrophthalazinedione sodium in the solutions was determined by spectrophotometric method at an absorption maximum of 294 ± 2 nm using formula:

$$x = \frac{D_x \times 10 \times 500}{D_0},$$

where D_x is optical density of the test solution, D_0 is optical density of the control sample, 10 is concentration (in mg/mL) of aminodihydrophthalazinedione sodium control solution, 500 is the volume (in mL) of the test solution.

Statistical processing of the results was done using Microsoft Office Excel 2010 software.

RESULTS

TTS application in each series of experiments lasted for 24 hours. The quantity of drug substance in the patches was 100 mg and 20 mg for the antidote for carbon monoxide and the immunomodulator, respectively.

The quantity of drug substance remaining in the patch after detachment was examined for both test substances.

Thus, the quantity of immunomodulator in TTS after application was 8.4 ± 2.8 mg. Consequently, ~11.6 mg of the drug entered the skin from the patch during 24 hours of the experiment. According to the results of the study of pharmacokinetics of aminodihydrophthalazinedione sodium in the blood of a rabbit during percutaneous administration, it was found that the time to reach a plateau in drug concentration on the pharmacokinetic curve was about 4 hours [7].

We made the assumption that the skin can accumulate the active substance that will continue to flow into the bloodstream even after removal of the transdermal patch from the skin and can compensate for the temporary delay in the onset of action of the transdermal dosage form. So, the time required to reach a constant concentration of the drug in the blood should be taken into account when replacing the patch in case of long-term use.

Table 1 shows the quantity of immunomodulator contained in the skin and subcutaneous fat of rabbits at different times after patch removal.

Table 1

Quantity of residual aminodihydrophthalazinedione sodium in the skin and subcutaneous tissue of rabbits at different times after removal of the transdermal patch

Object of study	Quantity of drug substance after patch removal (mg)			
	Immediately (n = 3)	At 4 hours (n = 3)	At week 1 (n = 3)	At week 2 (n = 2)
Skin	0.51 ± 0.02	0.10 ± 0.002	0.013 ± 0.005	0.0021 ± 0.0004
ST	0.006 ± 0.003	0.005 ± 0.002	0.005 ± 0.001	0.0013 ± 0.0005

As shown in Table 1, 0.516 mg of aminodihydrophthalazinedione sodium was present in the skin flap that had been in contact with the TTS for 24 hours immediately after its detachment. Over the next two weeks, there was a decrease in the quantity of the drug substance in the skin, with a significant decrease to 0.41 mg occurring in the first 4 hours. This value may be therapeutically significant in the case of transdermal administration, given the small daily dose of Galavit® (25 mg, orally) [8].

The results obtained during the study of the content of the immunomodulator in the skin should be taken into account when developing a regimen for the aminodihydrophthalazinedione sodium TTS.

A similar series of experiments was performed for TTS with the antidote for carbon monoxide. The quantity of active substance in the bis(1-vinylimidazole-N) zinc diacetate TTS after application in a rabbit was 28.1 ± 4.3 mg. Thus, about 70 mg entered the skin from the dosage form. About 1 mg active substance bis(1-vinylimidazole-N) zinc diacetate was present in the skin flap

that had been in contact with the patch for 24 hours immediately after its detachment (Table 2).

Table 2

Quantity of residual bis(1-vinylimidazole-N) zinc diacetate in the skin and subcutaneous tissue of rabbits at different times after removal of the transdermal patch

Object of study	Quantity of drug substance after patch removal (mg)			
	Immediately (n = 3)	At 4 hours (n = 3)	At week 1 (n = 3)	At week 2 (n = 2)
Skin	0.92 ± 0.01	0.89 ± 0.03	0.63 ± 0.02	0.19 ± 0.05
ST	0.06 ± 0.03	0.06 ± 0.02	0.05 ± 0.08	0.04 ± 0.03

Four hours after TTS removal, the quantity of the drug substance in the skin and subcutaneous fatty tissue remained virtually unchanged, in contrast to the result obtained at the same time point in skin examination after application of the immunomodulator TTS. At week 1 and 2, the quantity of the antidote decreased slightly to ~0.7 mg and ~0.25 mg, respectively. Thus, the quantity of active ingredient eliminated from the skin per week, 0.3–0.4 mg, is negligible compared to the required daily dose of the drug (120 mg orally) and may not have a significant therapeutic effect [9].

Three weeks after removal of the transdermal therapeutic system for both drugs, the quantity of active substance in the skin was at the lower limit of sensitivity of the quantitative methods used.

CONCLUSION

This work examined the residual amounts of the immunomodulator and antidote for carbon monoxide in animal skin and in transdermal therapeutic systems 24 hours after application. For transdermal application of aminodihydrophthalazinedione sodium, the skin can be a drug depot and prolong the effect of this drug even after the transdermal patch had been removed, compensating for the temporary delay in the onset of the next patch. No such effect was found in the case of bis(1-vinylimidazole-N) zinc diacetate, which is apparently due to the different solubility of the studied drugs in the biotissue.

So, when conducting preclinical studies of transdermal delivery systems with the aim of developing the dosage form application scheme, possible accumulation of drug substance in the skin layers in concentrations that have a therapeutic effect should be taken into account.

The authors declare no conflict of interest.

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