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## ORIGINAL ARTICLE

# RAPID INFORMATIVE SCREENING OF NANO-ALAPTIDE AS POTENTIAL TRANSDERMAL PERMEATION ENHANCER OF ACETYLSALICYLIC ACID AND PARACETAMOL

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**Summary**

This investigation deals with the affection of permeation of acetylsalicylic acid and paracetamol applied in the system propylene glycol-water 1:1 through full-thickness pig ear skin by alaptide that was applied in nanonized form as a potential chemical penetration enhancer. Alaptide, (S)-8-methyl-6,9-diazaspiro[4.5]decan-7,10-dione, is the original Czech compound. The application of nanonized alaptide significantly enhanced the permeation of both drugs through the skin. Enhancement ratios in the studied time interval 0.5-2.0 h varied from 1.11 to 17.70 for acetylsalicylic acid and from 6.83 to 19.83 for paracetamol.

*Key words: alaptide; nanoparticles; chemical penetration enhancer; acetylsalicylic acid; paracetamol*

## INTRODUCTION

Transdermal administration of drugs represents an excellent alternative to conventional pharmaceutical dosage forms. The advantages of transdermal administration include, above all, good pharmacokinetic properties of application systems and a very simple and painless application. At present transdermally applicable drugs include steroid hormones, antipsychotics, anodynes, various (non)-steroidal anti-inflammatory drugs or drugs affecting cardiovascular system [1−3].

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One of the major disadvantages of transdermal application is the possibility of local skin irritation or allergization by an active substance or an excipient. Other disadvantages include variable intra and interindividual absorption depending on the skin condition and, partly, on the site of application. Long-term transdermal application on the same site can damage the skin by affecting its microflora and enzymes [2−4].

Another disadvantage is longer time of effect onset connected with the need to overcome the skin barrier, because the application of transdermal drug delivery faces the problem of insufficient or no penetration of the active pharmaceutical substances through the skin. The skin consists of three basic functional layers: the upper layer (epidermis), corium (dermis), and subcutaneous tissue (hypodermis). The most important is the outermost layer of epidermis, the horny layer (stratum corneum), which is in fact

the skin barrier, and the penetration/permeation through this least permeable layer is a limiting proces [2−7].

Chemical permeation/penetration enhancers (CPEs) represent one of the approaches solving this critical issue. CPEs are special pharmaceutical excipients that interact with the skin components to increase penetration of drugs from the topical dosage forms to blood circulation. Several different chemical structures were evaluated as penetration enhancers, and various potential sites and modes of action were identified for them [2,5−7].

Alaptide (Figure 1) can be classified as a new potential CPE, because it was observed that under specific conditions alaptide is able to modify penetration/permeation/absorption of the compounds through the skin. Alaptide is an original Czech compound prepared by Kasafirek *et al.* in the 80s of the 20$^{th}$ century with a significant skin curative activity without any observed toxicity, which is helpful in reducing possible skin irritant/injurious effects of the penetrating compounds. As alaptide was designed as an analogue of melanocyte-stimulating hormone release-inhibiting factor (MIF), it is able to influence the creation and function of keratinocytes. The permeation of alaptide through full-thickness pig ear skin was evaluated, and on the basis of these results the application of nanonized alaptide (NALA) was suggested, as permeation of alaptide in this form through the skin was limited [8−11].

The aim of this study is to investigate the application of NALA as a potential CPE. Within a rapid informative screening the effect of nanonized alaptide on permeation of model non-steroidal anti-inflammatory drugs (NSAIDs) and analgetic/antipyretic drugs, namely acetylsalicylic acid and paracetamol (Figure 1), through full-thickness pig ear skin using a Franz cell from propylene glycol-water system (1:1) was estimated. NSAIDs were chosen as one of the most frequently applied classes of drugs and are characterized by gastrotoxicity, especially at long-standing per oral administration [12]. Acetylsalicylic acid from NSAIDs as well as paracetamol from the close class of analgetics/antipyretics were selected as model penetrants due to the fact that according to experimentally determined permeability coefficients using hairless mouse skin neither acetylsalicylic acid (Kp = $1.836\times10^{-3}$ cm$^2$/h [13]) nor paracetamol (Kp = $0.280\times10^{-3}$ cm$^2$/h [14]) rank among the preferred drugs for transdermal administration [1,15].

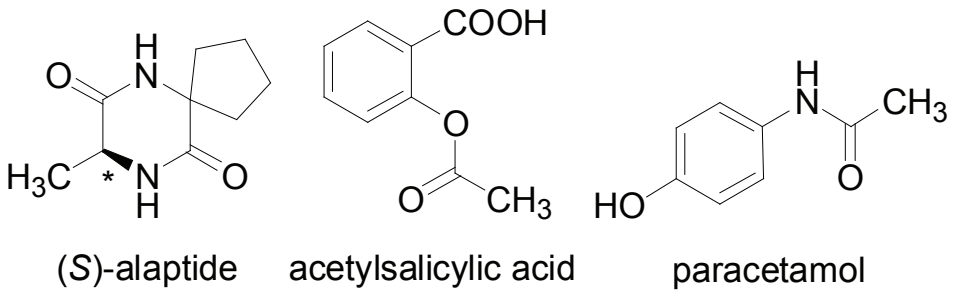


**Figure 1.** Structures of (*S*)-alaptide and tested compounds.

## MATERIAL AND METHODS

### *Preparation of nanonized alaptide*

The suspension of alaptide, synthesized according to Kasafirek *et al.* [16], (30 g), polyvinylpyrrolidone (30 g), and purified water (240 mL, during milling was diluted by addition of additional 150 mL) was initially mixed for 12 h at ambient temperature and then filtered through a mill sieve. The milling procedure was performed using a nanomill NETZSCH (Germany) with glass beads (0.3 mm); the rotor speed was 986 rpm; the pump speed was 30 rpm; the temperature in the grinding chamber was within 17–20 °C. The rotor speed was increased to 1500 rpm after 6 h of milling. The total time of milling was 57.5 h. The content of alaptide in the suspension was 38.76 g/L (determined by RP-HPLC [11]). The particle size of the prepared nanonized alaptide was measured by Sympatec NANOPHOX 0138 P (Germany), and the particle size $x_{90}$ was 770 nm.

*In vitro transdermal permeation experiments performed using Franz diffusion cell*

Skin samples were obtained from porcine ear. Full-thickness ear skin was cut in fragments and stored at −20°C until utilized. Skin samples were slowly thawed (at 4 °C overnight and then at ambient temperature) before each experiment. The penetration enhancing effect of NALA was evaluated *in vitro*, using a vertical Franz diffusion cell (SES – Analytical Systems, Germany), with a donor surface area of 0.6359 $cm^2$ and receptor volume of 5.2 mL. The skin was mounted between the donor and receptor compartments of the Franz diffusion cell with the epidermal side up. The receptor compartment was filled with phosphate buffered saline (pH 7.4) and was maintained at 34±0.5 °C, using circulating water bath. The receptor compartment content was continuously stirred using a magnetic stirring bar. The skin was kept in contact with the receptor phase for 0.5 h prior to the experiment. Acetylsalicylic acid, and paracetamol were obtained from Sigma-Alddrich. The solution (1%) of each of the drugs in propylene glycol-water system (1:1) was mixed with NALA (1% related to drug) and applied to the skin surface as a donor sample, and the donor compartment of the cell was covered by Parafilm®. Control samples were prepared in the same manner without NALA. Samples (0.5 mL) of the receptor phase were withdrawn at four pre-determined time intervals (30, 60, 90, and 120 min), and the cell was refilled with an equivalent amount of fresh buffer solution. A minimum of five determinations was performed using skin fragments from a minimum of 2 animals for each compound, and the data was expressed as means ± SD. The samples were immediately analysed by HPLC.

*Evaluation of samples*

The analysis of samples was performed using an Agilent 1200 series HPLC system, equipped with a diode array detection (DAD) system, a quaternary model pump, and an automatic injector (Agilent Technologies, Germany). Data acquisition was performed using ChemStation chromatography software. A Zorbax Eclicpse XDB C18 5 μm, 3.5×150 mm (Agilent, USA) chromatographic column was used for both compounds. The total flow of the column was 0.5 mL/min, injection 10 μL, column temperature 30 °C, and sample temperature 10 °C. The detection wavelength of 225 nm was chosen, the time of analysis was 10 min. A mixture of MeOH (HPLC grade, 95.0%) and H2O (HPLC – Mili-Q Grade, 5.0%) was used as a mobile phase for paracetamol. A mixture of MeOH (HPLC grade, 75.0%) and $H_2O$ (HPLC – Mili-Q Grade, 25.0%) was used as a mobile phase for acetylsalicylic acid. The retention time ($t_R$) of acetylsalicylic acid was 6.8±0.05 min, the limit of detection (LOD) was 151 ng/mL, and the limit of quantification (LOQ) was 503 ng/mL. The retention time ($t_R$) of paracetamol was 3.6±0.04 min, the limit of detection (LOD) was 118 ng/mL, and the limit of quantification (LOQ) was 354 ng/mL.

**Table 1.** Cumulative permeated amounts $Q_t$ per unit area [μg/$cm^2$] of acetylsalicylic acid (ASA) and paracetamol (PAR) from propylene glycol-water (1:1) without and with nanonized alaptide (NALA) as enhancer of transdermal penetration achieved in *in vitro* transdermal permeation experiments using Franz diffusion cell. $Q_t$ values are expressed as mean ± SD (n = 5 experiments). The means followed by different letters are significantly different at P = 0.05. The analysis was performed for two studied systems (ASA, PAR; n =5).

| Time [h] | | Cumulative permeated amounts $Q_t$ [μg/$cm^2$] | |
|---|---|---|---|
| | | ASA | PAR |
| 0.5 | drug | 24.1±3.1$^a$ | 8.2±0.4$^a$ |
| | drug+NALA | 164.7±4.0$^c$ | 9.1±0.9$^b$ |
| 1.0 | drug | 27.7±1.7$^{ab}$ | 7.9±0.5$^a$ |
| | drug+NALA | 421.0±2.6$^d$ | 52.1±2.9$^c$ |
| 1.5 | drug | 28.4±1.6$^b$ | 8.7±0.8$^{ab}$ |
| | drug+NALA | 494.1±3.8$^e$ | 105.8±4.0$^d$ |
| 2.0 | drug | 28.0±1.6$^b$ | 9.3±0.8$^b$ |
| | drug+NALA | 555.3±3.6$^f$ | 164.6±3.8$^e$ |

*Statistical evaluation*

All experiments were carried out 5-fold. Data were expressed as means ± SD. The differences were evaluated by a one-way analysis of the variance (ANOVA) test completed by Bonferroni's multicomparison test (ORIGIN PRO7). The differences were considered significant at P = 0.05. The independent variables and responses (cumulative permeated amounts and enhancement ratios) of all the samples were analysed using ORIGIN PRO7.

## RESULTS AND DISCUSSION

The skin permeation experiments were performed using static Franz diffusion cells [17], and full-thickness pig ear skin was selected for *in vitro* evaluation of permeation. This tissue is a suitable *in vitro* model of human skin [18,19], because porcine skin has proved to be histologically and biochemically similar to human skin, and it has been used in numerous studies [20].

The permeation of drugs through full-thickness pig ear skin without and with 1% (w/w related to drug) NALA was tested from propylene glycol-water system (1:1) as a donor vehicle, because most of the studies involved the use of propylene glycol (PG) or its mixture with water or ethanol as a donor vehicle. Previous studies indicated that PG by itself (or a PG/water co-solvent system) does not interfere with the membranes, but exhibits a synergistic effect in combination with other penetration enhancers [21−23]. All the results are listed in Table 1.

As both tested drugs are administered at pains/fever, and they should act as soon as possible, all experiments were performed only in the time range from 0.5 to 2.0 h. The values obtained from the permeation experiments were expressed as the cumulative permeated amount of the drug ($Q_t$ [μg]) per unit of skin surface area (0.6359 $cm^2$), see Table 1. Due to the short time interval of experiments steady-state permeation flux and lag time cannot be calculated (this was not the aim of the experiments), but according to the presented results it is evident that NALA can be successfully applied as a CPE. The enhancement ratios (ERs) were calculated as the ratios of cumulative permeated amount of the drug with alaptide and without alaptide (ER = $Q_t$ (with alaptide)/$Q_t$ (without alaptide), see Table 2.

**Table 2.** Enhancement ratios (ERs): $Q_t$ (with NALA)/$Q_t$ (without NALA) of acetylsalicylic acid (ASA) and paracetamol (PAR) related to transdermal permeation. ERs values are expressed as mean ± SD (*n* = 5 experiments). The means followed by different letters are significantly different at P = 0.05. The analysis was performed for both drugs (n =5).

| Time [h] | ERs = $Q_t$ (+NALA)/$Q_t$ | |
|---|---|---|
| | ASA | PAR |
| 0.5 | 6.83±1.03[a] | 1.11±0.15[a] |
| 1.0 | 15.20±1.10[b] | 6.59±0.15[b] |
| 1.5 | 17.40±1.12[c] | 12.16±1.14[c] |
| 2.0 | 19.83±1.03[d] | 17.70±1.86[d] |

The results of the one-way analysis of the variance (ANOVA) test complemented by the Bonferroni's multicomparison test are presented in Tables 1 and 2, where differences were considered significant at P = 0.05. Considerable differences between cumulative permeated amount of the drugs alone and after NALA addition were found and these differences were statistically significant at P = 0.05 in every monitored time point, see Table 1. Within investigated time interval 0.5-2.0 h enhancement ratio values showed a time-dependent increase and differed significantly from each other for both investigated drugs at P = 0.05, see Table 2.

Based on the data presented in Table 2 it can be concluded that alaptide exhibited enhancement activity since considerably higher permeation enhancement was observed for acetylsalicylic acid (ER from 6.83 to 19.83) and paracetamol

(ER from 1.11 to 17.70). That means that the contribution on NALA to the enhanced skin permeation of these drugs is significant. As mentioned above this is an interesting result, because in terms of physico-chemical properties neither acetylsalicylic acid nor paracetamol are drugs suitable for permeation according to their permeability coefficients.

The structure of alaptide can be classified as a hybrid between the derivatives of urea and 2-pyrrolidone, therefore the supposed mechanism of enhancement action can be as follows. As an urea-like derivative it can demonstrate moisturizing effect of the *stratum corneum* [6,7,22], and, on the other hand, as a 2-pyrrolidone-like derivative it can exhibit interactions preferentially in the keratin region [5,6]. Confirmation of the mechanism of action of alaptide will be the aim of our following investigation.

The results of the presented rapid screening and the above discussed facts may indicate utilization of nanonized alaptide as a CPE and in the development of transdermal therapeutic systems (TTS) or similar skin-applicable compositions. A TTS combining acetylsalicylic acid with NALA (that acts as a CPE and simultaneously has skin curative effect) could be used by patients with ischemic disease. A similar idea about transdermal delivery systems for acetylsalicylic acid has been discussed recently [24−27]. Also application of paracetamol in a TTS can avoid first-pass hepatic exposure and metabolism via portal circulation and accelerate assembly of action [14,28,29].

## CONCLUSION

The ability of alaptide in nanonized form to enhance permeation of model NSAIDs and analgetics/antipyretics acetylsalicylic acid and paracetamol through porcine skin was examined using a Franz cell within rapid preliminary *in vitro* screening. Nanonized alaptide applied in concentration 1% w/w related to the amount of the drug influenced permeation of drugs from the system propylene glycol-water (1:1). It was found out that in this small concentration alaptide can affect the drug level in the body in time, and formulations can be used for both local and/or systemic administration. Enhancement ratios of drugs with the application of nanonized alaptide in the studied time interval 0.5-2.0 h varied from 1.11 to 17.70 for paracetamol, and from 6.83 to 19.83 for acetylsalicylic acid, thus it can be finally stated that their enhanced permeability is caused by the effect of alaptide. These results for hydrophilic molecules, such as paracetamol and acetylsalicylic acid, indicate feasible utilization in transdermal delivery systems, therefore this issue will be investigated in detail. As found out, alaptide demonstrates skin curative effect; it can be used with benefit in combination with the drugs in various transdermal therapeutic systems, in which it can act as an enhancer as well as a skin protecting substance.

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