International Journal of Pharmaceutics, 87 (1992) 67–72

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IJP 02848

In vitro penetration of the dopamine D2 agonist N-0923 with and without Azone

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(Received 28 October 1991)
(Modified version received 17 February 1992)
(Accepted 18 March 1992)

Key words: N-0923; Aminotetralin; Dopamine D2 agonist; Azone; Penetration, in vitro; Rat skin; Penetration enhancement

Summary

The penetration of the dopaminergic D2 agonist *S*(−)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin (N-0923) was evaluated in vitro on full-thickness rat skin, using the Franz diffusion cell. The drug was tested as the N-0923 · HCl salt as well as the N-0923 base. The penetration-enhancing effect of Azone was studied in a vehicle concentration of 5%, or after pretreating shaven rat skin in vivo, at several time intervals before the in vitro experiment, with a solution containing 1 or 5% Azone. From the vehicle containing 60% ethanol, 20% propylene glycol and 20% water, the extent of penetration through rat skin was relatively low for the base and even lower for the salt. The introduction of Azone into the vehicle in a final concentration of 5% resulted in a 6-fold decrease in penetration for the free base. Nevertheless, penetration of the salt increased about 12-fold in extent during the first 12 h, after which it declined to almost zero. The lag time was reduced from 13 to 5 h. Pretreatment of rat skin in vivo with an ethanol-propylene glycol-water solution containing 5% Azone resulted in a 12-fold increase in flux for the salt, a 2-fold increase in flux for the base and a decrease in lag time to about the same values for both N-0923 · HCl and N-0923 base. In vivo pretreatment of rat skin with a solution containing 1% Azone had no significant effect on the flux and lag time vs controls. This study indicates that Azone may have potential in facilitating the transdermal application of N-0923, principally through pretreatment of the skin to enhance absorption. This may allow it to circumvent its considerable oral first-pass metabolism.

Introduction

Recently, Swart and De Zeeuw (1992) have demonstrated that the bioavailability of the dopaminergic D2 agonist *S*(−)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin (N-0923), after oral and intraperitoneal administration, is very low (0.5 and 8%, respectively). In vitro (Swart et al., 1991) and in vivo studies (Gerding et al., 1990; Swart and De Zeeuw, 1992) have indicated that the drug is extensively metabolized, mainly to the glucuronide conjugate, especially in the gut and liver. Therefore, the use of ester prodrugs (Den Daas et al., 1990) or alternative routes of administration should be considered in order to circumvent this first-pass metabolism.

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In the present study, we investigated the penetration of N-0923 · HCl and N-0923 base through full-thickness rat skin in vitro. In order to increase the delivery of N-0923 through the skin, the enhancing effect of Azone was evaluated. This was performed by adding Azone to the dosing vehicle, or by pretreatment of rat skin in vivo with a solution containing Azone.

Materials and Methods

Chemicals and reagents

The drug *S*(−)-2-(*N*-propyl-*N*-2-thienylethylamino)-5-hydroxytetralin hydrochloride (N-0923 · HCl) and 1-dodecylazacycloheptan-2-one (Azone) were obtained from Whitby Research (Richmond, VA, U.S.A.). The enantiomeric purity of N-0923 was determined by the method of Gerding et al. (1989) and was found to be at least 99.9%. The pK_a of the tertiary amine group was 7.9. The free base was prepared by dissolving an adequate amount of the HCl salt in a saturated solution of sodium hydrogen carbonate and extracting it with ether. The ether layer was dried over anhydrous sodium sulfate and then evaporated to dryness. The residue, an oil, was weighed, dissolved in ethanol to a final concentration of 65 μmol ml^{-1} and stored at −20°C. No degradation was observed for the base over a period of at least 6 months. Tritiated N-0923 was obtained from Du Pont de Nemours (Boston, MA, U.S.A.) with a specific activity of 80.6 Ci mmol^{-1} and a radiochemical purity of at least 95%, as checked by HPLC. The tritium labels were present in the propyl side chain. The internal standard (IS) 2-(*N*-propyl-*N*-2-*p*-fluorophenylethylamino)-5-hydroxytetralin hydrochloride was kindly donated by Horn and co-workers, Department of Medicinal Chemistry, University Centre for Pharmacy, Groningen, The Netherlands. The compound was found to be at least 98% pure according to HPLC and TLC.

1-Octanesulfonic acid (sodium salt) was supplied by Eastman Kodak (Rochester, NY, U.S.A.). Propylene glycol (PG) was purchased from Brocacef (Maarssen, The Netherlands). All other chemicals were of analytical reagent grade and were obtained from Merck (Darmstadt, Germany). Throughout the study de-ionized water was used (Milli-Q, purification system, Millipore, Bedford, MA, U.S.A.).

Chromatography

The chromatographic system consisted of a model SP 8800 HPLC pump (Spectra Physics, San Jose, CA, U.S.A.), and a model 460 autosampler fitted with a 500 μl loop (Kontron Instruments, Basel, Switzerland). Detection was performed using an amperometric detector, with a working potential of 0.75 V, model AMOR, equipped with a glassy carbon electrode (Spark Holland, Emmen, The Netherlands). Peak heights were recorded with a 3396A reporting integrator (Hewlett Packard, Avondale, PA, U.S.A.).

The separation was performed using a 150 × 3.8 mm i.d. column packed with 4 μm Nova Pak C18 (Waters, Milford, MA, U.S.A.). The isocratic mobile phase used for the separations contained 65% 50 mM sodium phosphate pH 5.5, 35% acetonitrile, 3.5 mM 1-octanesulfonic acid and 5 mM sodium chloride. After filtering the eluent through a 0.20 μm type RC-58 membrane filter (Schleicher and Schuell, Dassel, Germany), the mobile phase was degassed in an ultrasonic bath for 15 min. During the measurements the eluent was continuously de-aerated with helium. The flow rate was 1.0 ml min^{-1}. The column and detector were thermostatted at 25°C with a circulating water bath (Wilton, De Meern, The Netherlands), to minimize fluctuations in background current. When not in use, the chromatographic system and the detector were continuously flushed with eluent at a flow rate of 0.2 ml min^{-1}.

Animals

Male Albino Wistar rats (CDL, Groningen, The Netherlands), weighing 240–260 g, were used. 2 days before the experiments the hair of the abdominal region was carefully removed with an electric razor without damaging the skin. For the experiments in which pretreated skin was used, the shaven skin was treated in vivo as follows: At

five time points, 49, 42, 25, 18 and 2 h before the in vitro experiment, the rats were fixed on their backs and 0.5 ml of a solution containing ethanol-propyleneglycol-water (60 : 20 : 20 v/v), with or without 1 or 5% Azone at the expense of water, was spread on the skin (20 cm^2) by means of a glass rod. 1 h before starting the in vitro experiments rats were anaesthetized with ether, the skin excised, and the animals killed.

Penetration experiments

The penetration experiments were performed using Franz-type diffusion cells (Franz, 1975). The skin was mounted between the cell compartments with the stratum corneum towards the donor compartment. The receiver chamber had a volume of 4.3 ml and was filled with isotonic phosphate-buffered saline, containing 136 mM NaCl, 2.68 mM KCl, 1.47 mM KH_2PO_4, and 8.08 mM Na_2HPO_4, with a final pH of 7.4. It was kept at 37°C by circulating water through an external water jacket. After 30 min of equilibration of the skin with the receptor fluid, 0.2 ml of the drug solution, containing 2.7 μmol N-0923 · HCl or N-0923 base, was applied uniformly on the stratum corneum by means of a pipet. The donor compartment was covered with parafilm to prevent evaporation of the solvent. With an effective diffusion area of 1.35 cm^2, a dose of 2 μmol cm^{-2} N-0923 was thus applied. The experiments were run for 26 h. The receptor phase was continuously stirred by means of a spinning bar magnet, at 350 rpm (Multipoint HP 15, Variomag, München, Germany). Receptor fluid samples of 0.25 ml were withdrawn from the receptor compartment at various time intervals. The cell was refilled with receptor fluid.

The experiments were performed with N-0923 · HCl and N-0923 base and the influence of the penetration enhancer Azone was investigated as follows:

Azone 5% in the dosing solution The dosing solution consisted of ethanol-propylene glycol-water-Azone (60 : 20 : 15 : 5 v/v). The control dosing solution was ethanol-propylene glycol-water (60 : 20 : 20 v/v).

In vivo pretreatment of the abdominal skin of the rat with a solution containing either 5 or 1% Azone The pretreatment solutions consisted of ethanol-propylene glycol-water-Azone 60 : 20 : 15 : 5 (v/v) or 60 : 20 : 19 : 1 (v/v), respectively. Controls were pretreated with ethanol-propylene glycol-water 60 : 20 : 20 (v/v). Dosing was carried out using a solution containing the salt or the base in ethanol-propylene glycol-water 60 : 20 : 20 (v/v).

The stability and possible metabolic conversion of N-0923 during the study were investigated by means of tritiated N-0923 · HCl. The dosing solution was spiked with 11 kBq [^{3}H]N-0923, and the first of the two experiments described above was performed, without the use of Azone.

Back-diffusion of the drug from the receptor compartment towards the skin was studied as follows:

The receptor compartment was filled with receptor fluid spiked with 5 nmol ml^{-1} N-0923 · HCl and 2.3 kBq [^{3}H]N-0923 · HCl. Untreated rat skin was mounted on the diffusion cell, and samples were withdrawn from the receptor fluid, at various time intervals, for the analysis of both unlabeled and radioactive N-0923.

Sample preparation

To 100 μl of receptor fluid 100 μl of water containing the IS were added and the mixture was vortex mixed. 30 μl of the diluted sample was injected on the HPLC system. Concentrations were determined by means of a standard curve and were corrected for the cumulative amount of drug that had permeated the skin. Each experiment was repeated at least four times. The cumulative amount of N-0923 released with time per unit area (nmol cm^{-2}) was evaluated. From each individual curve the flux and lag time were calculated. All data given in Tables 1 and 2 are expressed as means ± S.D.

Aliquots of 100 μl obtained from the radioactive permeation were mixed with 2 ml Picofluor (Packard, Groningen, The Netherlands). The radioactivity was measured in a Beckman LS 1800 scintillation counter (Beckman Industrial Corp., La Habra, CA, U.S.A.). Quenching was corrected by the H-number method and the concentration of N-0923 was calculated after subtracting the background.

TABLE 1

Penetration of N-0923 through non-pretreated skin

	Azone in vehicle (%)	Flux (nmol cm^{-2} h^{-1}) (mean ± S.D.)	Lag-time (h) (mean ± S.D.)	n
N-0923·HCl	0	0.9 ± 0.6	13.4 ± 2.4	6
	5	11.2 ± 2.8 [a,b]	4.6 ± 0.7 [b]	6
N-0923 base	0	2.6 ± 0.6	6.7 ± 2.3	4
	5	0.4 ± 0.3 [b]	4.3 ± 1.5	4

[a] Up to 12 h, then declining to almost zero.
[b] Change in flux or lag time as a result of the Azone pretreatment is statistically significant, at $P < 0.05$ (Student's t-test).

Results and Discussion

Azone in the vehicle

The N-0923 penetration data (flux in nmol cm^{-2} h^{-1} and lag time in h) are shown in Tables 1 and 2, and Figs 1–4. The drug was dissolved in 60% ethanol, 20% propylene glycol and 20% water, in order to achieve sufficient solubility for both N-0923 · HCl and N-0923 base. Propylene glycol was selected in order to enhance the permeability of the skin (Wotton et al., 1985; Barry, 1987). Ethanol has also been proposed to increase skin permeability of polar solutes via solvent channel pathways (Ghanem et al., 1987). N-0923 · HCl showed poor solubility in isotonic phosphate-buffered saline of about 1 mg ml^{-1}. However, 2.7 μmol of the drug (corresponding to about 0.9 mg) were on the skin, and no solubility

TABLE 2

Penetration of N-0923 salt and base through skin pretreated in vivo with Azone

	Azone in solution (%)	Flux (nmol cm^{-2} h^{-1}) (mean ± S.D.)	Lag-time (h) (mean ± S.D.)	n
N-0923·HCl	0	1.1 ± 0.4	9.8 ± 2.0	7
	1	1.6 ± 1.4	8.5 ± 1.3	6
	5	13.4 ± 4.1 [a]	5.7 ± 2.2 [a]	7
N0923 base	0	5.1 ± 2.2	10.2 ± 1.7	7
	1	5.7 ± 1.9	8.1 ± 1.7 [b]	6
	5	11.5 ± 4.3 [a]	6.9 ± 1.6 [c]	7

[a–c] Statistically significant at $P < 0.05$ (Student's t-test).
[a] 5% compared with 0 and 1%, [b] 1% compared with 0%.
[c] 5% compared with 0%.

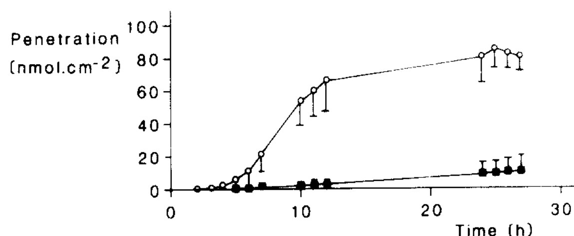

Fig. 1. Cumulative penetration of N-0923·HCl through non-pretreated rat skin. (■) Vehicle containing 60% ethanol, 20% PG, 20% water; (○) vehicle containing 60% ethanol, 20% PG, 15% water, 5% Azone.

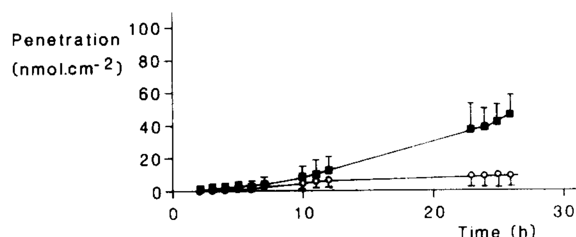

Fig. 2. Cumulative penetration of N-0923·base through non-pretreated rat skin. (■) Vehicle containing 60% ethanol, 20% PG, 20% water; (○) vehicle containing 60% ethanol, 20% PG, 15% water, 5% Azone.

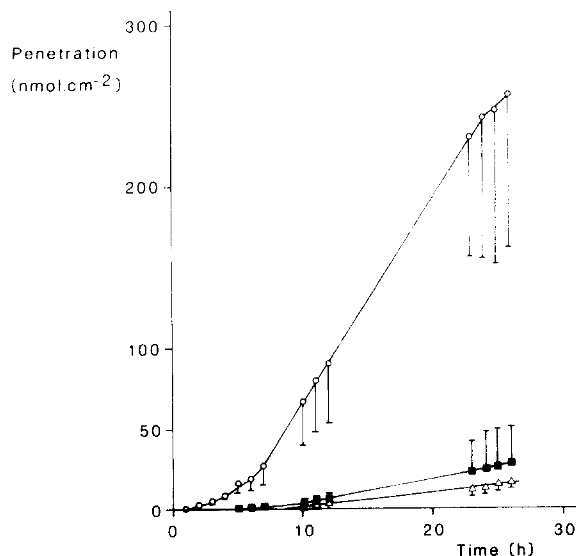

Fig. 3. Cumulative penetration of N-0923·HCl through pretreated rat skin. The dosing vehicle contains no Azone. (△) Pretreatment with 60% ethanol, 20% PG, 20% water; (■) pretreatment with 60% ethanol, 20% PG, 19% water, 1% Azone; (○) pretreatment with 60% ethanol, 20% PG, 15% water, 5% Azone.

problems were encountered in the receptor compartment due to its relative large volume of about 4.3 ml.

The penetration of N-0923 · HCl from a non-Azone containing vehicle through non-pretreated rat skin was found to be 0.9 ± 0.6 nmol cm^{-2} h^{-1}, with a relatively long lag time of about 13 h. The penetration could be initially enhanced by adding 5% Azone, at the expense of water, to the vehicle. The flux was increased 12-fold, and the lag time reduced to about 5 h as compared to the control solution. However, after 12 h the flux declined remarkably to almost zero. We have no explanation for this phenomenon.

Penetration of the more lipophilic N-0923 base from a non-Azone containing vehicle resulted in a mean flux of 2.6 ± 0.6 nmol cm^{-2} h^{-1}, which is 3-fold greater than that found for N-0923 · HCl. Also, a shorter lag time of about 6.7 h was observed. However, the penetration of N-0923 base could not be stimulated by addition of Azone to the vehicle. In fact, a negative effect, namely, a 7-fold decrease in flux (0.4 ± 0.3 nmol cm^{-2} h^{-1}) and no change in lag time were observed, as compared to controls. The decrease in penetration may be explained by the improved solubility of the base and decrease in the thermodynamic activity of the Azone containing vehicle.

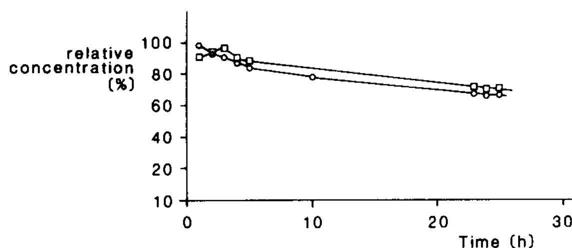

Fig. 5. Diffusion of N-0923·HCl from the receptor phase towards the skin [(○) N-0923, (□) tritiated N-0923]. Relative concentrations of N-0923 in the receptor phase plotted vs time.

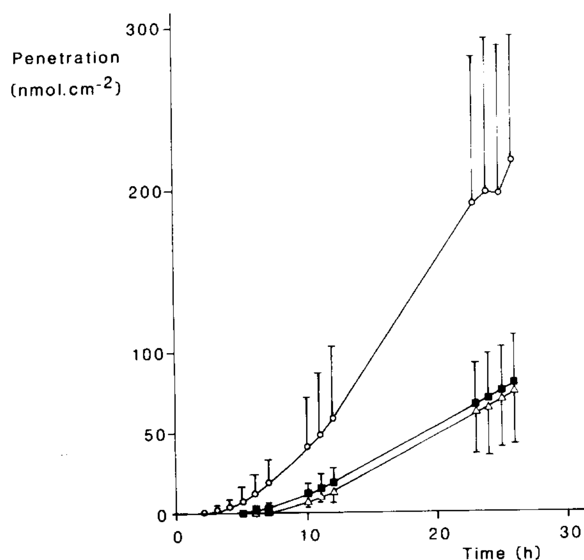

Fig. 4. Cumulative penetration of N-0923 base through pretreated rat skin. The dosing vehicle contains no Azone. (△) Pretreatment with 60% ethanol, 20% PG, 20% water; (■) pretreatment with 60% ethanol, 20% PG, 19% water, 1% Azone; (○) pretreatment with 60% ethanol, 20% PG, 15% water, 5% Azone.

In vivo pretreatment with Azone

The penetration of N-0923 · HCl and N-0923 base through rat skin was pretreated, in vivo with a solution containing 60% ethanol, 20% propylene glycol, and 20% water, resulted in similar fluxes and lag times, as compared to penetration without pretreatment. Therefore, it can be concluded that pretreatment with propylene glycol and ethanol had no enhancing effects under the conditions used.

Also, in vivo pretreatment of rat skin with a solution containing 60% ethanol, 20% propylene glycol, 19% water, and 1% Azone changed the fluxes and lag times for neither N-0923 · HCl nor N-0923 base, as compared to the controls. However, the addition of 5% Azone, at the expense of water, to the pretreatment solution resulted in a 12-fold increase in flux for N-0923 · HCl and a 50% reduction of the lag time. After 26 h, approx. 15% of the dose had reached the receptor compartment. Under the same conditions, the flux of N-0923 base doubled, with a reduction of the lag time by 50%. As can be seen in Table 2, the 5% Azone pretreatment in this case resulted in flux and lag time values that were very similar for the salt and the base.

Drug stability

The penetration of N-0923 · HCl from a vehicle containing 60% ethanol, 20% propylene glycol, and 20% water (v/v), which was spiked with 11 kBq tritiated N-0923, resulted in the same parameters for flux and lag time, as compared to the study in which no radioactivity was used. This indicates that the drug is not being metabolized and thus is stable under the conditions used.

Back-diffusion

In a study in which 5 nmol ml^{-1} N-0923 · HCl was added to the receptor fluid, 5% appeared to back-diffuse into the non-pretreated skin in 26 h. Similar results were obtained using 5 nmol ml^{-1} N-0923 spiked with 2.3 kBq $[^3H]$N-0923. Fig. 5 shows the decrease in concentration of N-0923 · HCl from the receptor compartment. This may indicate that larger amounts of the drug penetrate into the skin than found in the receptor solution and that the release from the skin to the receptor compartment is dependent on the drug's partition coefficient between the skin and receptor phase.

Conclusions

The fluxes through rat skin of both N-0923 · HCl and N-0923 base could be increased some 2-fold (base) or 12-fold (HCl salt) by pretreating the skin in vivo with a solution containing 5% Azone. Pretreatment also reduced the lag times considerably. These findings support the possibility that N-0923 may be delivered via the transdermal route and therefore may be an attractive alternative to obtain therapeutically relevant systemic plasma concentrations. Therapeutic N-0923 plasma levels seem virtually impossible via the oral route because of the major first-pass metabolism of this dopaminergic drug.

Acknowledgement

This research was supported in part by Whitby Research, Richmond, VA, U.S.A.

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