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The effects of Azone and capsaicin on the permeation of naproxen through human skin

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Abstract

The permeation of naproxen through excised human skin and isolated perfused rabbit ear skin has been determined. It was found that both Azone and capsaicin enhanced the permeation with an enhancement ratio of up to 4-fold. The magnitudes of the effect were similar in human and rabbit skin. The permeation of naproxen from a saturated solution of the drug through skin pre-treated with Azone was similar to that from a commercial preparation (Naprosyn). In the perfused rabbit ear experiments the presence of capsaicin had no effect on the vasodilatation of the blood vessels, inferring that the penetration enhancement was a direct result of capsaicin influencing the barrier function of the skin. Structural similarities between Azone and capsaicin were seen using molecular graphics.

Keywords: Azone; Capsaicin; Naproxen; Penetration enhancers; Skin absorption

1. Introduction

Naproxen is a non-steroidal anti-inflammatory agent (NSAID) whose permeability through skin has been determined previously and shown to be 2.9×10^{-3} cm/h (Degim et al., 1998). It is a compound that has the potential to be delivered topically for local action and one that could be promoted with the appropriate use of permeation enhancers.

Another potential treatment for joint inflammation is capsaicin since it is known as an antagonist to substance P (Goodman and Gilmans, 1990). It is also known to deplete sensory neurons containing substance P, calcitonin gene-related peptide (CGRP) and neurokinins (Bertrant et al., 1993). It can contract smooth muscle and has excitatory effects on thin, primary afferent neurons (Skibata et al., 1998). It is a medicinally accepted material

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found in Capsicum, which has been grown as a medicinal plant in Europe, Asia and Africa for many centuries and has some topical effectiveness (Zhang and Li Wan Po, 1994).

It may be possible to combine a traditional NSAID, such as naproxen, with capsaicin to produce a more effective topical pharmaceutical product. The chemical structure of capsaicin is shown in Fig. 1. It is interesting to compare the dimensions of capsaicin with those of Azone, also depicted in Fig. 1. The structures were energy minimized using ACD software (Advanced Chemistry Development, Toronto, Canada) and interatomic distances measured. The distance along the longest axis of both compounds is very similar ≈ 17 Å. Azone is a well-documented penetration enhancer (Hadgraft et al., 1993). This substance appears to act by reducing the diffusional barrier of the stratum corneum by inserting itself into the structured lipids located in the intercellular channels. The fluidity of the microenvironment of the lipids is reduced and diffusion improved. It is possible that capsaicin itself may also be a, hitherto unreported, penetration enhancer. The chemical structures have some similarities—e.g. a ring at one end of a long alkyl chain—but the partition behaviour of the two are very different. Capsaicin has a predicted logP of 3.31 whereas that of Azone is 7.82. Azone would be anticipated to concentrate in the stratum corneum lipids far more than capsaicin. It is probable that the percutaneous absorption of capsaicin is much better than Azone and it may be anticipated to enhance absorption and also penetrate sufficiently to elicit a pharmacological effect.

Enhancer effects of both capsaicin and Azone on naproxen were determined in vitro using human tissue and ex vivo using a perfused rabbit ear model. Histological evaluations on human skin were also conducted.

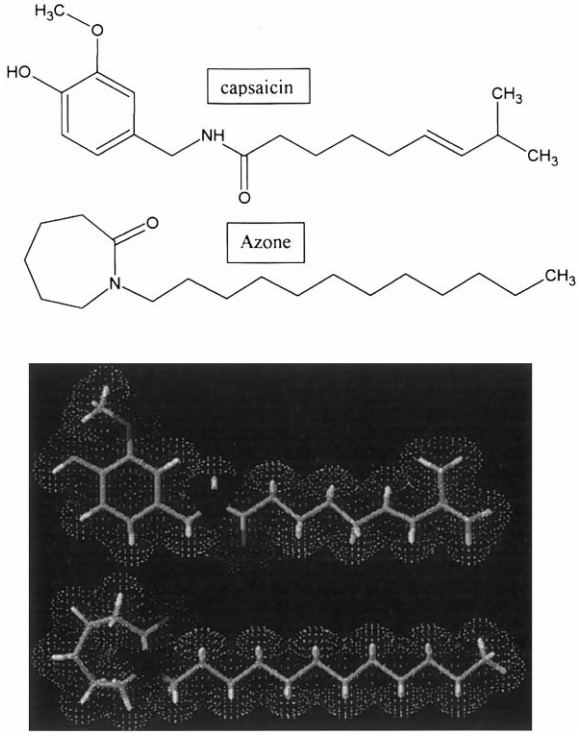

Fig. 1. The chemical structures of capsaicin and Azone together with energy minimized three-dimensional representations.

2. Materials and methods

Capsaicin was purchased from Fluka (Switzerland). Azone was donated by Whitby Pharmaceuticals (Virginia, USA). All buffer components and high performance liquid chromatography (HPLC) solvents were standard analytical grade. Commercial preparations of naproxen were purchased: Inaprol (BilimAyazaga Köyü yolu, Maslak, Istanbul, Turkey), Naponal (Munir Sahin, Çavusoglu Mah., Sanayi Cad., Istanbul, Turkey), Naprosyn (Abdi Ibrahim, Kore Sehitleri Cad., Istanbul, Turkey).

2.1. In vitro permeation studies

Full-thickness, female, human skin was obtained following abdominal surgery. Underlying fatty tissue was removed by blunt dissection and, if not used immediately, the skin was stored frozen, at -15°C for not longer than 2 weeks. The skin was mounted in all-glass Franz-type diffusion cells and thermostatted such that the skin surface was at 32°C and the stirred receptor medium at 37°C. The cross-sectional area of the cells was 1 cm^2. Saturated solutions of naproxen

were prepared by stirring excess drug in pH 5 buffer overnight. Phosphate buffered saline pH 7.4 (≈ 2 ml) was used as the receptor medium. Five or six replicates were conducted for each treatment protocol. Samples were taken over a 48- or 72-h period and naproxen concentrations determined by HPLC. Removed receptor medium was replaced with fresh buffer.

2.1.1. Ex vivo experiments using isolated perfused rabbit ears

One week prior to the study the hair on the skin was removed using commercial depilatories. The animals were sacrificed under thiopental anaesthesia and the ears removed. Small PTFE tubes were inserted into the main artery of the ears. Aerated Krebs solution at 37°C was perfused through the ears at 1.5 ml/min. The conditions ensured the viability of the tissue over the time course of the experiment. Donor solution (5 ml) was applied in a plastic reservoir in close contact with 9 cm^2 of the skin. The plastic reservoir was sealed with petroleum jelly to prevent any leakage. The perfusate was collected and naproxen concentrations determined by HPLC.

2.2. Enhancer pretreatment strategy

Skin samples were pre-treated with 50 μl/cm^2 of 3% Azone or capsaicin in ethanol for 2 h prior to the diffusion experiment. During this time the ethanol evaporated from the unoccluded site.

2.3. Microscopy

Human skin samples were treated with and without Azone or capsaicin as above. They were fixed in paraffin wax and examined under a light microscope to determine any gross structural changes in the stratum corneum.

2.3.1. Vasodilator effect of Azone, capsaicin and alcohol

In the perfused rabbit ear experiments, any vasodilator effect of the formulation components was monitored using a pressure sensor and manometer connected in series with the PTFE tube and the artery of the ear. Phenylephrine (2 μM) was injected into the arterial tube to produce contraction, followed by 3 ppm alcohol or capsaicin or Azone. Any pressure changes were recorded.

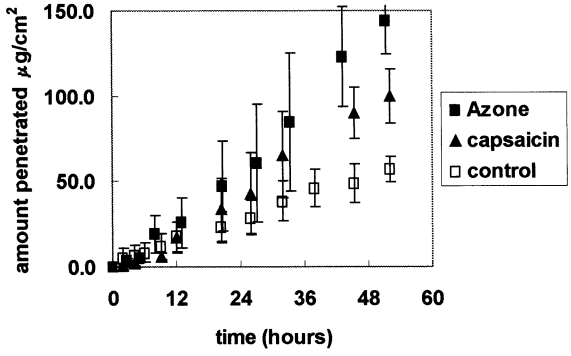

Fig. 2. The permeation of naproxen through full-thickness human skin. The effects of pre-treating the skin, compared to control ($n = 6$), with capsaicin and Azone (both $n = 4$) are shown. The data are presented with the S.E.M.

2.4. HPLC

The analysis of the naproxen was based on that by Degim et al. (1998) Briefly a C18 ODS (25 × 0.4 cm, particle size 0.5 μm) column was used with a mobile phase (flow rate, 1 ml/min.) consisting of acetonitrile (137.5 ml), water (112.5 ml), K_2HPO_4 (0.619 g) and orthophosphoric acid (1 ml). The naproxen was detected on a diode array detector at 230.9 nm.

3. Results and discussion

The effect of Azone and capsaicin on the permeation of naproxen through full-thickness human skin is shown in Fig. 2.

The steady-state fluxes, from Fig. 2, are 2.84 ± 0.11 (S.D.), 2.04 ± 0.08, 1.07 ± 0.03 μg/cm^2/h for, respectively, Azone, capsaicin and the control. The enhancer effects of capsaicin and Azone are roughly 2- and 3-fold, respectively. The enhancement ratio for Azone is similar to that found under similar circumstances for metronidazole (Wotton et al., 1985). Capsaicin does appear to have some enhancer properties of its own.

Diffusion through the perfused rabbit ear is shown in Fig. 3.

The fluxes of naproxen averaged over the 6 h are, respectively, for the control, capsaicin and Azone pre-treatment: 0.34 ± 0.03 (S.D.), 1.29 ± 0.1, 1.51 ± 0.04 μg/cm^2/h. Under these conditions, the enhancement ratios are capsaicin (3.8) and Azone (4.4). The effect is of a comparable magnitude to that found for human tissue. The overall fluxes of naproxen are lower than those found for human tissue. This is perhaps surprising since it is generally appreciated that rabbit tissue is more permeable than human tissue (Chowhan and Pritchard, 1978).

The amount of naproxen diffusing through the skin and into the perfusate will be a function of the rate of permeation through the different strata of the skin and its uptake into the perfusion fluid. If this latter step is not efficient it can have an influence on the determined flux. However, it is unlikely to be the dominant rate-controlling process since enhancement effects were observed.

The materials could influence uptake into the perfusate by acting as vasodilators. The experiments that determined the effects of ethanol, Azone and capsaicin on the vasodilatation of the blood vessels showed no effects when the materials were applied topically. After direct injection at a concentration of 3 ppm, all three agents appeared to have a small effect on reducing the intra-arterial pressure. It appears that the enhancers are acting on the barrier function of the skin and that uptake into the perfusion fluid is not the major rate-determining factor in the rabbit experiments.

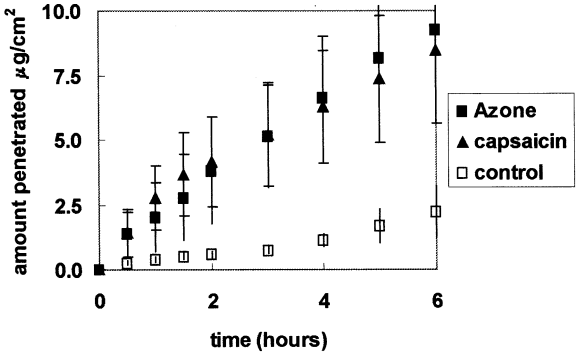

Fig. 3. Penetration of naproxen through perfused rabbit ear. The effects of pre-treating the skin, compared to control ($n = 6$), with capsaicin and Azone (both $n = 4$) are shown. The data are presented with the S.E.M.

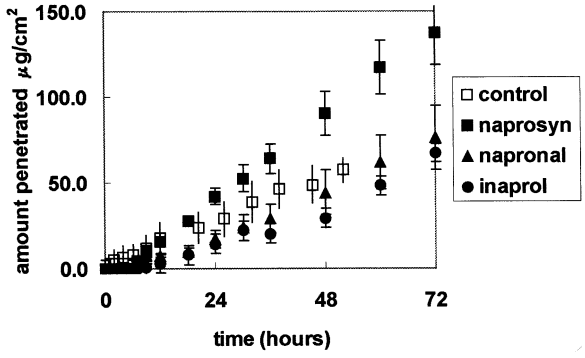

Fig. 4. A comparison between the in vitro permeation of naproxen through human skin from commercial preparations ($n = 5$, ± S.E.M.) and control (saturated solution of naproxen, $n = 6$, ± S.E.M.).

In the final part of the study, comparisons were made between a saturated solution of naproxen and commercially available topical preparations containing naproxen. These were Naprosyn, Napronal and Inaprol. Figs. 4 and 5 show, respectively, the amounts of naproxen permeating human and rabbit skin for the commercial preparations compared to the saturated solution of naproxen (control).

In the in vitro experiments using human tissue, Napronal and Inaprol behaved very similarly to

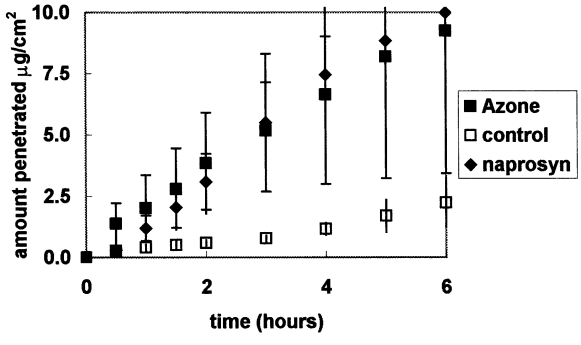

Fig. 5. A comparison between the permeation of naproxen through perfused rabbit ear for a control (saturated solution of naproxen ($n = 6$, ± S.E.M.), Azone pre-treatment with saturated solution ($n = 4$, ± S.E.M.) and Naprosyn ($n = 4$, ± S.E.M.).

the saturated solution of naproxen. Naprosyn appeared better than the control, and an analysis of variance shows that there is a significant difference between the control and the amounts of naproxen permeated at 72 h. It is possible that the higher flux from the Naprosyn is due to the formulation having naproxen dissolved in it close to its solubility limit. Also formulation components in the preparation (e.g. ethanol) will permeate the skin concurrently at high levels and will alter the solubility parameter of the skin lipids. Drug transfer will be enhanced as a result of the properties of the skin membrane being modulated.

Without a direct knowledge of all the formulation ingredients it is not possible to state precisely what enhancement strategies have been used. It is apparent that the topical formulation has been designed such that it has an enhancement capacity close to that observed for Azone (cf. Fig. 2).

The enhancement from Naprosyn was also examined in the perfused rabbit ear model and very similar results were established (see Fig. 5).

The microscopical evaluation of the skin sections showed no gross changes in the structural features of the stratum corneum for any of the treatment protocols. There was some evidence of skin thickening as a result of the treatment with Azone or capsaicin but the effects were not large. Any enhancement created by Azone or capsaicin was not thought to be as a result of structural damage to the outer layers of the skin.

4. Conclusions

The experiments have demonstrated that both Azone and capsaicin are capable of enhancing the permeation of naproxen through human and rabbit skin. The enhancement effects on the two species were very similar. The structural likeness (in terms of molecular size) suggests that the mechanisms are similar. It is thought that capsaicin will insert itself into the lipid bilayers within the intercellular channels and create disruption in their stacking. This will reduce the diffusional resistance of the intercellular domains. A consideration of the physicochemical properties of capsaicin suggests that this molecule will permeate the skin more readily than Azone. Since it acts as an enhancer for the NSAID and also has potential activity in its own right (lowering substance P), it may be possible to create topical analgesic formulations in which there are two active ingredients one of which, in addition, acts as an enhancer to its partner. It is probable that this could create synergistic effects and may create formulations that are more effective than capsaicin or naproxen alone.

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