

## ISOLATED PERFUSED RABBIT EAR MODEL FOR THE ASSESSMENT OF TRANSDERMAL DRUG DELIVERY

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**ABSTRACT:** Isolated perfused rabbit ear model together with full thickness human skin and PDMS membrane were used to investigate the penetration properties of naproxen and the enhancer activity of capsaicin. The effect of capsaicin was also compared with well known enhancer azone. Different amounts of chosen enhancers were applied to the skin surface before the experiment. Naproxen was chosen as a model penetrant and the quantity of penetrated naproxen was determined by HPLC. Commercially available naproxen gel formulation and an alternative formulation containing 3 % capcaisin were also studied and results were compared.

Penetrations were found to be increased when the skin was treated with azone and capsaicin. In perfused rabbit ear experiments, the enhancing mode of action of capsaicin is probably due to its chemical property and lipophilicity but not due to its vasodilatory action since the amount added into the formulation was given topically to the skin and no effects were observed on the perfusion pressure. However, intraarterial injection of those compounds decreased the pressure via their vasodilator effects.

The effects of capsaicin and azone on skin using microscope were also aimed to investigate in this study and treated skin samples were investigated under light and electron microscope. It was found that capsaicin caused some alterations on stratum corneum layer of the skin like azone therefore it was observed that capsaicin caused an enhanced penetration of naproxen through human skin.

In conclusion capsaicin was found to be a quite capable enhancer for skin penetration of drugs like the well-known enhancer, azone. The perfused rabbit ear model is found to be useful to study transdermal uptake of drugs and it can particularly be used during preclinical development studies. Although capsaicin was found to be a good penetration enhancer, the addition of the capsaicin to the gel formulation did not provide faster penetration.

[Key words: rabbit, capsaicin, transdermal, drug delivery]

### INTRODUCTION

The development of non-invasive mechanical and technical dermal drug delivery systems increasingly requires adequate and reproducible skin in-vitro assay models, which guarantee a high reliable predictive estimation of medico-chemical potential of a given system

in each state of development (1). The broad range of different model of drug application, ie. superficial intra or subcutaneous, requires an even complex assay model, which tends to be as close to physiology of the intact skin and associated subcutaneous tissues as possible. Some ex-vivo models have been proposed to

investigate skin penetration such as porcine ear or organ models and rabbit organ models.

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) whose permeability through full thickness of human skin has been determined previously and shown to be  $2.9 \times 10^{-3}$  cm 7h (2,3). Naproxen is a compound that has the potential to be delivered through skin locally and one that could be promoted with the appropriate penetration enhancers, because limited number of drugs possess the appropriate physico-chemical characteristics in appropriate formulation to allow them to cross the main barrier of the skin, the stratum corneum (4). The range of molecules that can be successfully delivered can be increased by the use of penetration enhancers that reduce the effectiveness of the barrier so that the flux of the drug reaches a clinically useful level (5). Capsaicin is a compound used for joint inflammation since it is known to be an antagonist to substance P (6) and depletes sensory neurons containing substance P, calcitonin gene-related peptide (7CGRP) and neurokinins (7). It contracts smooth muscle and has excitatory effects on thin, primary afferent neurons (8). It is a medically accepted compound found in a plant (known as Capsicum), which has been medicinal plant in Europe, Asia and Africa for many centuries and has some topical effectiveness (9). It may be possible to combine a traditional NSAID such as naproxen with capsaicin to produce a more effective topical gel or it may be possible to improve the penetration properties of NSAID from commercially available gel formulations. Azone is one of the best known penetration enhancers and it has been shown to be effective for a wide range of permeants. It is believed that Azone acts by increasing the fluidity of the compact lipid bilayers that lie between the keratinocytes of the stratum corneum (10). The molecular structure of capsaicin was found to be similar to that of azone. The present study was aimed to compare the penetration enhancing effect of azone and capsaicin. Penetration properties of naproxen through full thickness of human skin

and PDMS membrane were determined using Franz type of diffusion cells and results were compared. Capsaicin can increase the blood supply of dermal tissues by vasodilatation and perfused animal ear models have been proposed to investigate transdermal penetration of compounds (3,11,12), therefore perfused rabbit ear model was selected to investigate the effect of capsaicin on living dermal tissues. Naproxen in solution, commercially available gel formulation and gel containing capsaicin were used and penetration properties were investigated using perfused rabbit ear model. Human skin samples were pre-treated with Azone, capsaicin and alcohol and the changes on skin cells were investigated under light and electron microscope.

## MATERIALS and METHODS

Capsaicin was purchased from Fluka (Fluka Chemie Ag., CH-9471 Buchs, Schweiz), Azone was obtained from Welson research (1001 Health Sciences Road, West Irvine, California, 92715 USA). Commercially available gel formulation Naprosyn (Abdi Ibrahim, Kore Sehitleri Cad. No:19, Zincirlikuyu, 80300 Istanbul) was purchased from the market. An alternative gel formulation was prepared by adding 3% capsaicin to Naprosyn gel. All buffer components and HPLC solvents were of analytical grade.

Full thickness human skin samples were obtained from hospital shortly after the cosmetic operation. Healthy skin samples were selected and fatty tissues were removed by surgical blade. Full thickness skin samples were kept in a deep freezer (at  $-15^{\circ}\text{C}$ ) until use (for no longer than a week). Rabbit ears were obtained from freshly sacrificed rabbits.

Composition of Krebs' Solution in mmol/L:

NaCl	118
KCl	4.8
CaCl <sub>2</sub>	25
NaH <sub>2</sub> PO <sub>4</sub> ·2H <sub>2</sub> O	1.2
MgSO <sub>4</sub> ·7H <sub>2</sub> O	1.2

NaHCO<sub>3</sub>            24  
Glucose            11

HPLC: Waters Milipore (510 pump, 717 Plus Autosampler, 996 Photodiode Array Detector)

Infrared spectrophotometer: Shimadzu, IR-470.

Peristaltic pump: Desaga, STA Peristaltic pump.

Pressure sensor: Statham physiological pressure transducer.

Polygraph: Grass Model 79-C D.C. driver amplifier with EEG&Polygraph data recording system, Grass Instrument Co. Quincy, Mass., USA.

Electron microscope: EM-900 Carl Zeiss-Germany.

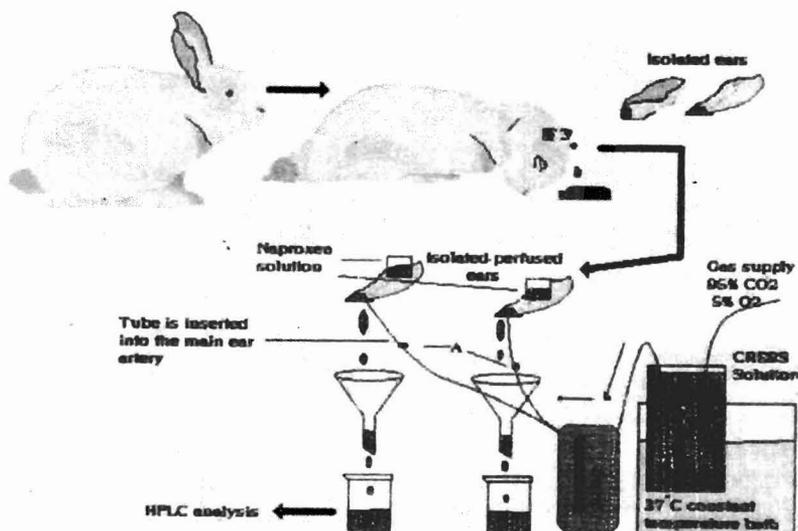
#### Permeation studies using full thickness human skin

The diffusion of Naproxen across full thickness human skin was studied using a series of static diffusion cells of the Franz design. Full thickness human skin was placed between the donor and receptor compartments of the diffusion cell, then, cell compartments were clamped. The cross sectional area was 1cm<sup>2</sup>. The joint of the cell was checked, if any leakage problem occurred, the process was repeated or the membrane was changed. The cells were placed on a magnetic stirrer in a water bath at 37°C. Saturated solutions of Naproxen were prepared by stirring excess solid overnight in the phosphate buffer (pH=5). Saturated solution of Naproxen was

placed in the donor chamber of the diffusion cell. The receptor solution was a phosphate buffer (pH=7.4). Diffusion cells were filled with 2 ml of receptor solution. Five or six replicate determinations were conducted for each treatment or experiment. Samples (about 0.5 ml) were taken from the receptor phase and analyzed by HPLC using the method outlined below.

#### Ex-vivo penetration studies using isolated perfused rabbit ear

All hairs on the ears were removed using depilatory creams. After a week, rabbits were sacrificed under thiopental anaesthesia and the ears were removed. Small PTFE tubes were inserted into the main artery of the ears. The tubes were then connected to the perfusion tube (Figure-1). Krebs solution at 37°C was perfused using a peristaltic pump. 95%CO<sub>2</sub> and 5%O<sub>2</sub> mixture was circulated through the Krebs solution to keep ears alive. Ears were perfused for an hour with the Krebs solution. A plastic reservoir with the diameter of 3-4 cm was used to apply Naproxen solution or gel to the ear surface. Plastic reservoir was placed on top of the ear surface and then joints were sealed with petroleum jelly to prevent solution leaking. 5 ml of naproxen solution or gel was put into the plastic reservoir. Perfusates were collected using funnels and then analyzed by HPLC. The flow rate of the perfusion was selected as 1.5 ml/min. Ears were observed to be swollen when higher flow rates were used.



**Figure-1:**The schematic illustration of the design of isolated-perfused rabbit model.

Determination of vasodilator effect of Azone, Capsaicin and Alcohol

Possible vasodilator effects of azone, capsaicin and alcohol were determined using a pressure transducer and manometer. The pressure transducer and manometer were connected to the tube just before the connection of arterial tube. The pressure changes were recorded to detect any vasodilator effect. Ears were perfused with Krebs solution for an hour for the equilibration and the pressure was observed to be constant.  $2 \times 10^{-6}$  M phenylephrine was injected to the arterial tube to ensure viability and response of the tissues. 3 ppm alcohol, azone and capsaicin were then injected to the arterial tube and the pressure changes were recorded.

HPLC method for Naproxen

Column: Reverse phase C18, ODS (25x0.4 cm, particle size 5(m)  
 Flow rate: 1 ml/min.  
 Mobile phase: Acetonitrile (137.5 ml), Water (112.5ml),  $\text{KH}_2\text{PO}_4$  (0.619g.), Orthophosphoric acid (1ml)

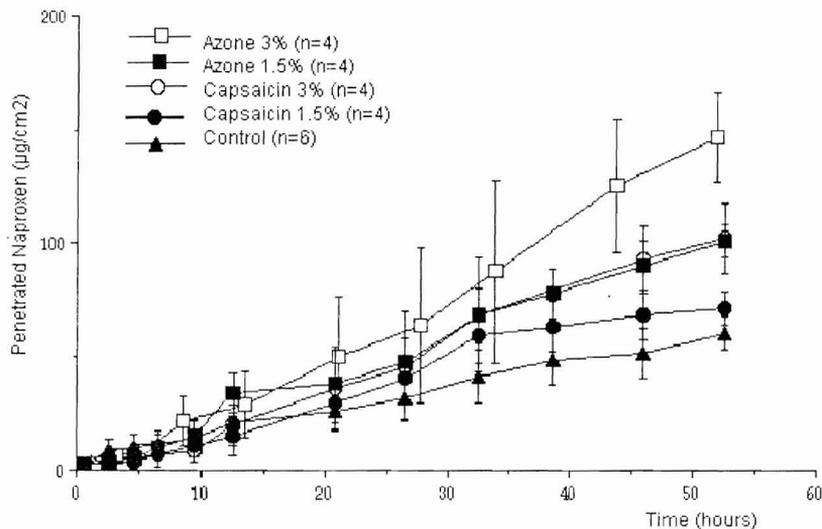
Naproxen was detected at 230.9 nm. The amount of naproxen was calculated according to the previously obtained calibration curve.

Statistic

A computer program called Instat (Graphpad software Version 2.04a) for statistical analysis.

**RESULTS**

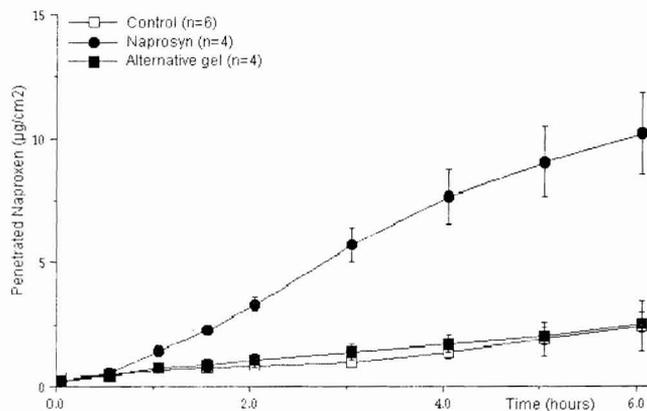
Diffusion experiments were performed using Franz type diffusion cells to understand the enhancer activity of chosen enhancers. Skin samples were pre-treated with 1.5 - 3% azone and 1.5 - 3% capsaicin solution in alcohol ( $50 \mu\text{l}/\text{cm}^2$ ) for two hours. After evaporation of alcohol, naproxen solutions were applied. Samples were collected and analyzed with HPLC. Figure-2 shows the penetration profiles of naproxen solution through full thickness of human skin with or without enhancer application.



**Figure-2** The effects of enhancers on penetration profiles of Naproxen through full thickness of human skin (Error bars represent SEM).

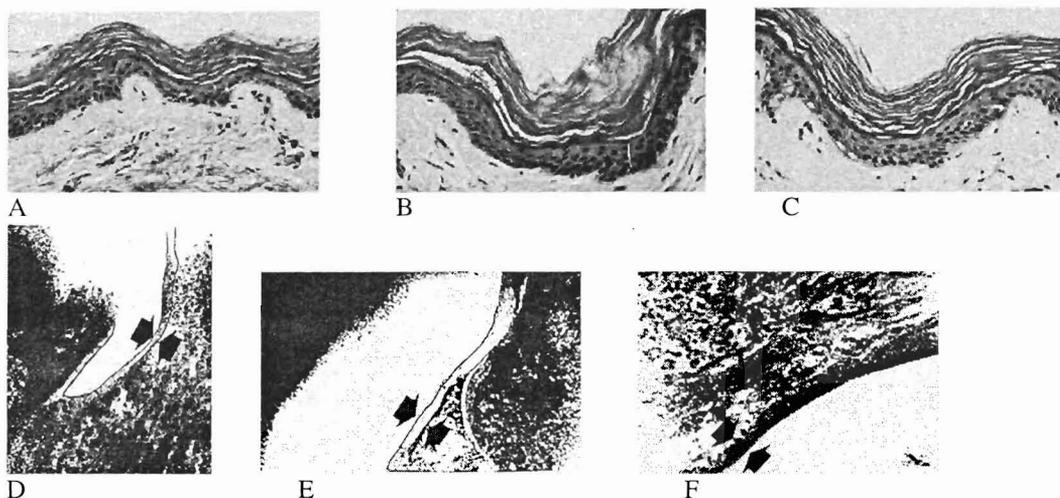
The pressure changes were recorded during the perfused rabbit ear experiment. Although pressure changes were observed after intraarterial administration of phenylephrine and 3 ppm of enhancers, no changes on the pressure were observed when alcohol, azone and capsaicin were applied to the ear skin surface. Further permeation studies were performed using perfused rabbit ear

experiment with commercially available naproxen cream. The penetration profiles were compared with naproxen solution. Capsaicin was found to be quite good enhancer therefore, a new cream of Naprosyn with 3% capsaicin was prepared. Figure-3 shows the penetration profiles of naproxen solution and alternative gel formulation compared with the commercially available cream.



**Figure-3** The penetration profiles of naproxen from the gel formulations through rabbit ear (Error bars represent SEM).

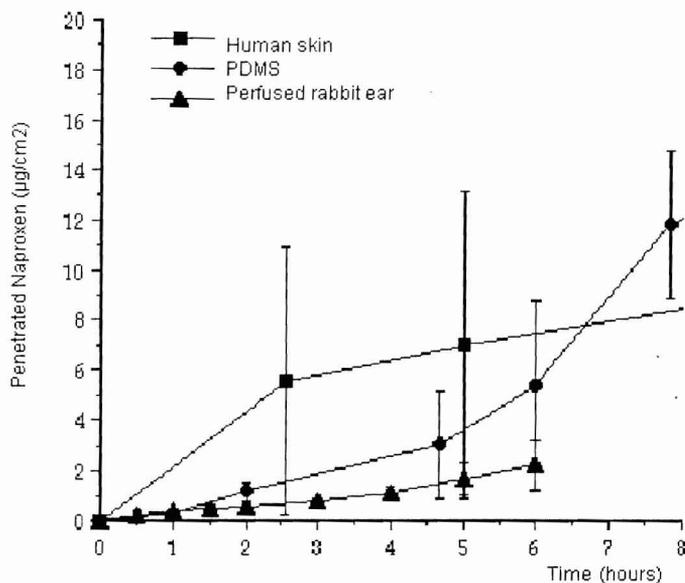
Human skin samples were pre-treated with azone, capsaicin and alcohol and the changes on skin cells were investigated under light and electron microscope. Figure-4 shows the changes on skin layer cells after enhancer application.



**Figure-4** Appearance of skin layers A (Control), after treatment with azone (B) and capsaicin (C) (x400); skin layer cells D (control), after treatment with azone (E) and capsaicin (F) (x85000).

Franz type of diffusion cells were used to determine the penetration properties of Naproxen through PDMS membrane. Figure-5 shows the penetration profiles of naproxen

through full thickness of human skin, PDMS membrane and rabbit ear skin. PDMS membrane gave close results with human skin.



**Figure-5** Penetration of naproxen through different membranes (Error bars represent SEM)

## DISCUSSION

The penetrations were found to be increased when skin samples were pre-treated with azone and capsaicin. Azone is very well known enhancer but the enhancer effect of capsaicin was also detected and proved using light and electron microscope in this study. Using a computer program, the octanol/water partition ( $\log K_o/w$ ) of azone and capsaicin were calculated to be 6.2-7.82 and 3.04-4.00 respectively.  $\log K_o/w$  value indicates the lipophilicity of the molecule. The higher values indicate that the molecule can stay among the skin lipids. In the enhancer case, enhancers have to have enough partition coefficients to stay among skin lipids. The  $\log K_o/w$  of capsaicin values is smaller than azone, so, the reason to obtain lower penetration was found to be related with  $\log K_o/w$  values of the enhancer.

The pressure changes were observed after intraarterial administration of phenylephrine and 3 ppm of enhancers. These results indicate that enhancers were pharmacologically active and ears were alive during the experiment, application of the enhancer to skin surface did not alter the blood perfusion. Although alcohol and enhancers slacken the muscle of blood vessels, using them for transdermal application was found to be ineffective.

Penetration profiles of naproxen solution and alternative gel formulation were compared to that of commercially available cream after perfused rabbit ear experiment. Unexpectedly, the penetration of naproxen from alternative gel was observed to be less than the others. The reason was thought to be some interactions between capsaicin and naproxen. The possible interaction was investigated using IR spectroscopy. The IR spectrum of naproxen, capsaicin and their physical mixture (1/1 molar ratio) were studied. The bands at  $1700\text{ cm}^{-1}$ ,  $1765\text{ cm}^{-1}$  are due to C=O stretching vibrations. In the formulation of alternative gel, presence of alcohol may cause removal of water from the molecule of capsaicin and naproxen. An extra peak was observed at  $1684\text{ cm}^{-1}$ . This

may be due to the formation of ester like structure. The extra bands at  $1370\text{ cm}^{-1}$  and  $3030\text{ cm}^{-1}$  may also be an indication of chemical interaction between two molecules.

Human skin samples were pre-treated with azone, capsaicin and alcohol and the changes on skin cells were investigated under light and electron microscope. The homogenisation of the stratum corneum layer of the skin was observed when skin was pre-treated with enhancers. The thickening of the horny layer of the skin and disappearance of some desmosomes and the formation of larger vacuoles were also observed after investigation under electron microscope. There were no changes after alcohol pre-treatment.

In conclusion, although the vasodilator effects of capsaicin and azone were demonstrated, capsaicin may mainly act as an enhancer when applied to the skin surface by means of its chemical properties and lipophilicity like azone. The vasodilator effect of capsaicin was found not to be significant. The mechanism of effects of capsaicin and azone on the stratum corneum using light and electron microscope were first established in this study and they were found to be act in similar way by altering the stratum corneum structure.

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