

Original Article

Effect of local controlled heat on transdermal delivery of nicotine

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Skin permeability and local blood perfusion are important factors for transdermal drug delivery. Application of heat is expected to enhance microcirculation and local perfusion and/or blood vessel permeability, thus facilitating drug transfer to the systemic circulation. In addition, heating prior to or during topical application of a drug may facilitate skin penetration, increase kinetic energy, and facilitate drug absorption. The aim of the present study was to investigate whether application of controlled local heat would enhance transdermal delivery from the nicotine patch mounted on the upper arm of ten healthy non-smoking male Caucasian subjects. Local skin perfusion was monitored using Laser Doppler Imaging (LDI) at baseline (32 °C) and following application of local controlled heat (43 °C) on the upper arm, where the patch was placed. The residue of the nicotine patches was then examined by High-Performance Liquid Chromatography (HPLC) to indicate the uptake of nicotine from the patch due to the local controlled heat. Controlled heat application (43 °C) caused significant cutaneous hyperaemia (up to 9 folds increase in skin perfusion) with an increase in nicotine uptake (up to 13 folds). The method was well tolerated without causing any pain or discomfort. These data suggest that controlled heat application, which is a simple, non-invasive method, can significantly enhance local skin perfusion and drug uptake from patches.

Keywords: Transdermal, nicotine, patch, local heat, skin perfusion

Introduction

Oral drug delivery is the most desirable and preferred method of administering therapeutic agents for their systemic effects [1]. However, first pass metabolism in the gut and liver greatly reduces the bioavailability of an oral dosage form [2]. In addition, some specific conditions such as unconsciousness, nausea and vomiting and difficulty of swallowing, limit the oral use of a given drug. Transdermal administration (e.g. application of a topical cream or a cutaneous patch) is considered as an alternative way. The most important barrier for transdermal route of administration is penetrating the stratum corneum (SC) [3]. Unique structure of SC only permits lipophilic drugs with small molecular weight to penetrate via passive diffusion driven by the gradient between the high concentration in the delivery system (e.g. a patch) and the zero concentration in the skin. Once diffusion through the multiple layers of skin is achieved,

the drug molecules are absorbed by the capillary plexus and are then transferred into the general circulation by local blood vessels [4, 5].

The rate and amount of transdermal absorption into the systemic circulation depend on several factors, including skin permeability and local tissue perfusion [6]. Several studies have shown that heat increases the skin permeability, body fluid circulation, blood vessel wall permeability, rate-limiting membrane permeability and drug solubility followed by increases kinetic energy and drug absorption [7]. It has been reported that in subjects wearing a nicotine patch sitting in a sauna, skin blood flow and transdermal drug uptake significantly elevated [8]. Similar cases have been reported on elevated absorption of nitroglycerine from patches during exposure to high ambient temperature [9].

We have recently reported that short duration of local application of controlled heat (43 °C/60

sec) causes significant regional cutaneous hyperaemia (increase in skin perfusion) without inducing any pain or discomfort in different body regions in both females and males [10]. Repeated application of controlled heat (every 5 min) on cutaneous perfusion also indicated that it is possible to maintain the vasomotor reaction at a constant level without significant changes over time [10].

Thus, the aim of the present study was to investigate whether application of local controlled heat (43°C) would enhance the transdermal uptake from nicotine patch in healthy males. We used nicotine patches as those are available without prescription and one of the widely used patches. However, one can consider similar paradigm to possibly enhance drug uptake from e.g. analgesic patches in a controlled manner to overcome breakthrough pain.

Materials and methods

Subjects and study design

Ten healthy male volunteers (age 23 ± 2 years) were recruited for this study through on-campus advertisements at Aalborg University, Denmark. Initial screening involved recording of demographic information and a review of medical history. The use of alcohol and caffeine, cold and hot drinks as well as smoking were prohibited 2 hours prior to the test and during the study. No subject had a past or present history of current systemic or skin diseases and none were taking any medication. Application of topical creams, lotions or cosmetics on the test sites was not allowed. Subjects with tattoos or scars at the site of patch application (upper arm) and smokers (more than five cigarettes per day) were excluded. Written informed consent was obtained from all participants prior to the study. The study protocol was approved by the local ethics committee (N-20090044). The study was conducted in accordance with the Declaration of Helsinki at the research laboratories of Aalborg University, Denmark.

The study was designed to investigate the uptake of nicotine from the nicotine patch following the application of local controlled heat (43°C). The subject relaxed on a comfortable bed at a supine position and exposed the test site. All experiments were done following acclimatization to the environment, in a quiet room with a

constant temperature (23-24°C).

Nicotine patch

The applied patch was Nicorette 5 mg/16 hours, batch number KM109, produced by McNeil AB, Norrbroplatsen 2, Helsingborg, Sweden. Subjects were asked to wear a nicotine patch for duration of 30 minutes. The patch was not heated continuously for 30 minutes as our preliminary tests showed continuously heating can potentially melt the glue on the patch. After 30 minutes the patch was removed and kept refrigerated for further analysis of the patch residue.

Heat application

The Medoc PATHWAY Pain & Sensory Evaluation System (Medoc Ltd. Advanced Medical Systems, Ramat Yishai, Israel) was used for controlled local heat application. The specific stimulation parameters were adjusted using the PATHWAY software (v. 4.4). The stimulation program was manually configured before initiation of the experiments. The applied parameters are summarized in **Table 1**. A 3.0 x 3.0 cm ATS thermode was utilized. The contact probe was attached to

Table 1. Controlled local heat application: Program configuration

Probe Type	ATS
Program Type	Ramp and Hold
Baseline (°C)	32
Trigger Configuration	Manual
Destination Temperature (°C)	43
Destination Rate (°C/sec)	2
Return Rate (°C/sec)	1

the skin by means of an elastic Velcro band.

Local controlled heat (43°C) was delivered for short periods of time in a repeated manner i.e. heat was delivered every 5 min for 30 min and every heat application was followed by a gap of 5 minutes. The heating rate toward the destination temperature was set to 2°C/sec, to prevent a sensation of burning. Heating was applied by placing the flat heating side of the thermal probe on top of the patch. As the thermode and skin was only separated by the thin layer of the nicotine patch, the skin temperature was

Table 2. Configuration for Laser Doppler Imaging (LDI): Scanning parameters

Bandwidth (kHz)	0.25 - 15
Resolution (px)	116 x 70
Scan Area (cm x cm)	6.0 x 6.0
Scan Speed (ms/pixel)	4
Scan Distance (cm)	30

most likely equivalent to the thermode temperature (i.e. $43^{\circ}\text{C}\pm 0.2$).

Laser Doppler Imaging (LDI)

Scanning was performed using a Moor Laser Doppler Imager (Moor Instruments, Devon, United Kingdom). In this study the scanner setup was adjusted using the parameters listed in **Table 2**.

The scanning area, called the region of interest (ROI), was defined using a template. The ROI was a 6.0 x 6.0 cm area, which allowed a safety margin of 1.5 cm at either side of the 3.0 x 3.0 cm thermode, when applied in the center of the 6.0 x 6.0 cm area. The laser scanner was adjusted and calibrated to scan the ROI. Before and following every heat application, a laser scanning was performed. Laser scans were stored on computer's hard disk for off-line analysis of the profile and local changes of skin perfusion (moor LDI software (v. 5.3). Subjects were asked to keep their eyes closed while wearing special goggles during laser scanning.

Analysis of the patch residue

Nicotine residue in the patches was determined using HPLC which indirectly indicated the nicotine transdermal uptake from the patch.

Each patch was placed in 40 ml of Toluene (CHROMASOLV, for HPLC, Sigma-Aldrich), which dissolved the patch glue membrane and the sealed bottles were kept refrigerated (5°C) for maximum of one month. The patches were handled using gloves and without touching the adhesive side of the patch. The nicotine were then extracted into 60 ml buffer containing potassium dihydrogen phosphate (analytical grade, Merck), phosphoric acid 85% (Fluka Analytical) and water purified by milli-Q. A sample of the buffer was transferred to a test tube and centrifuged for 10 minutes at 3000 rpm. One ml was

then taken from the solution and transferred into another test tube and 4 ml of neutralizing solution was added. The sample was then centrifuged for 5 minutes at 3000 rpm and the aliquot was then analyzed by HPLC. A HPLC system (Dionex, P680 HPLC pump, ASI-100 Automated sample injector, UV-detector UVD170U, Chromeleon 6.80 software) equipped with a Phenomenex 100 Å, C-18, 150x4.60 mm, 5 microns column, combined with an ultraviolet detector set to a wavelength of 260 nm, was used. The extracted nicotine samples were analysed on the HPLC with a gradient eluent consisting of methanol 99.8% (J.T. Baker), acetonitrile (gradient grade for liquid chromatography, Merck) and a phosphate buffer (patent pending composition).

HPLC absorption spectra were used to obtain the nicotine residue. The integral of the nicotine spike was used to calculate the nicotine residue.

Statistical analysis

Mean blood flow comparisons and nicotine uptake were analyzed using *t*-test. The statistical evaluation was made by means of the SigmaPlot version 11.0 (SPSS Inc., Chicago, US). $P < 0.05$ was considered as significant.

Results

All subjects completed the study and no adverse reaction was reported. Only two subjects experienced itch at the site of the patch during the application of heat.

Vasodilatory response to local controlled heat

The application of local controlled heat significantly increased average skin perfusion over 30 minutes. The average of baseline skin perfusion (at 32°C) was 127.0 AU and reached to 1100.0 AU after application of controlled heat (at 43°C) ($p < 0.05$) which is equal to 9 folds increased in skin perfusion. The baseline measurement of blood flow, the average blood flow over 30 minutes of intermittent heating and the percentage increase in blood flow following local controlled heat are depicted in **Table 3**.

Nicotine uptake and patch residue

The application of local controlled heat signifi-

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Table 3. Individual and mean values of blood flow (arbitrary units) at 32 °C, averaged over 30 minutes of intermittent heating and percentage increase in blood flow.

Subject	Blood flow at 32 °C (Baseline)	Average blood flow over 30 min of intermittent heat	% increase in blood flow
1	113.8	1228.7	1079.7
2	134.8	1058.2	785.0
3	100.8	1151.7	1142.5
4	160.8	1131.2	703.5
5	103.1	1106.6	1073.3
6	214.3	1149.4	536.4
7	121.2	1407.3	1161.2
8	97.4	842.0	864.4
9	90.5	879.1	971.4
10	133.5	1045.9	783.5
Mean	127.0	1100.0	910.1

cantly increased the uptake of nicotine from patch. The average increase was 1322.9% or 13 folds ($p < 0.05$).

In order to calculate the increase in nicotine uptake, a baseline uptake was required. Based on the manufacturer claim, the patches had a $\pm 7\%$ variation in initial content. Seven percent variance is equal to 0.581 mg nicotine. The patches are designed to release 5 mg over 16 hours, which gives a release of 0.1563 mg over 30 minutes. The average nicotine residue in the patches was 6.233 mg which gives an average nicotine uptake of 2.067 mg, varying from 1.486 mg to 2.648 mg. Nicotine uptake for each subject and the percentage of increase is given in **Table 4**.

Blood flow and nicotine uptake data was tested for a correlation and none was found. **Figure 1** shows increase in blood flow (following application of local controlled heat) and increase in nicotine uptake. The measurements are based on initial patch nicotine content of 8.3 mg and a theoretical baseline nicotine uptake of 0.1563 mg.

Discussion

The present study utilized local controlled heat to enhance local skin perfusion and nicotine uptake from the patch. Our findings demonstrated that the application of local controlled

heat (43 °C, 5 min on, 5 min off, for total duration of 30 min) could significantly enhance the skin perfusion (up to 9 folds) with no associated pain or discomfort and increase the nicotine uptake (up to 13 folds) with no detectable damage to the patch. This finding may provide the basis for developing a standard method, which is capable of producing a stable and significant potentiating of skin perfusion to the drug application site when it is applied topically (e.g. patches).

Increased skin perfusion following the application of heat may be mediated through a variety of mechanisms including local and neural mechanisms such as activation of axon reflex and nitric oxide synthase (NOS) system [15, 16]. However, the exact mechanism needs to be determined. We applied local controlled heat of 43 °C, since this heat level is below the usual heat pain threshold in adults [17] and would not cause pain. Application of higher temperatures may cause pain and subsequently reduce the blood flow, as pain may constrict the blood vessels [18, 19].

Overall, we found a significant increase in nicotine uptake following the application of the controlled local heat. However, the variation in initial content of the patches made it difficult to find the exact amount of uptake. Elevation of skin perfusion, following the application of local heat, might wash the nicotine away from the

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Table 4. The individual and mean uptake of nicotine based on initial patch content and percentage increase in uptake. The percentage increase is based on a theoretical baseline uptake of 0.1563 mg

Subject	Nicotine uptake (mg) based on an initial patch contents of:			% increase in nicotine uptake based on an initial patch content of:		
	8.3mg	7.719mg	8.881mg	8.3mg	7.719mg	8.881mg
1	1.4878	0.9068	2.0688	952.2	580.4	1324.0
2	2.0202	1.4392	2.6012	1292.9	921.1	1664.8
3	2.2987	1.7177	2.8797	1471.2	1099.3	1843.0
4	2.3625	1.7815	2.9435	1512.0	1140.2	1883.9
5	2.1923	1.6113	2.7733	1403.1	1031.3	1774.9
6	2.3075	1.7265	2.8885	1476.8	1105.0	1848.7
7	2.3109	1.7299	2.8919	1479.0	1107.1	1850.8
8	2.1629	1.5819	2.7439	1384.2	1012.4	1756.1
9	1.3054	0.7244	1.8864	835.4	463.6	1207.3
10	2.2215	1.6405	2.8025	1421.8	1049.9	1793.6
Mean	2.0670	1.4860	2.6480	1322.9	951.0	1694.7

% Increase in Blood Flow and Nicotine Uptake

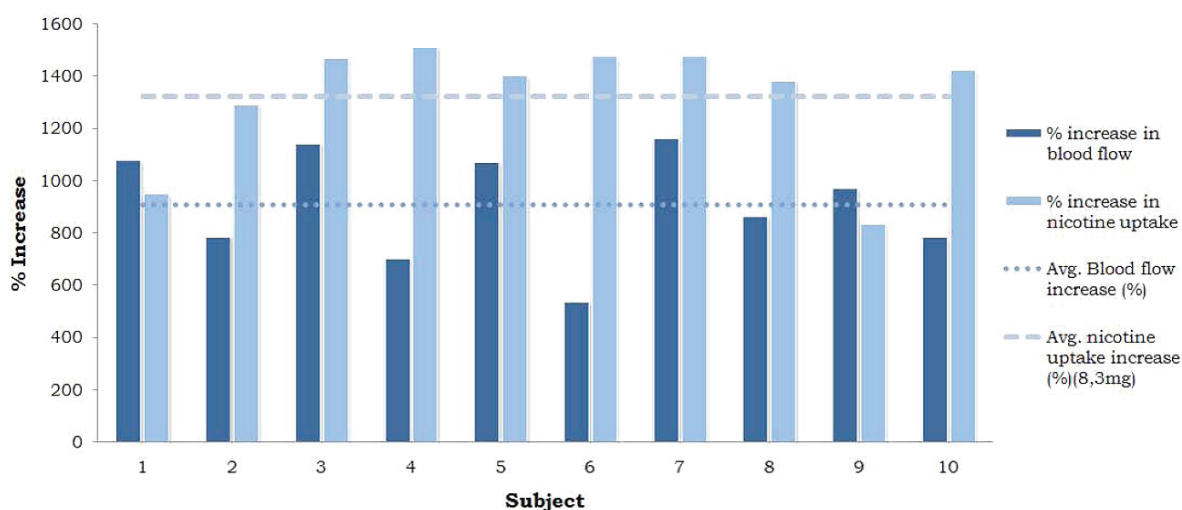


Figure 1. Percentage increase in blood flow for each subject, percentage increase in nicotine uptake for each subject, average percentage increase in blood flow and average percentage increase in nicotine uptake.

tissue at the site of the patch. This would subsequently increase the diffusion gradient and results in less residual nicotine in the patch as it has been shown in the present study. Other studies have shown increase of uptake from patches due to heat [9]. However, the amount of uptake can not be comparable since the ap-

plication of heat paradigms (local vs systemic) or drug is different from the present study [9, 20].

Absorption of drugs from cutaneous tissue depends on several factors, such as the quantity and composition of the tissue, capillary density,

vascular permeability, and the rate of vascular perfusion [5, 21]. Studies have demonstrated that clearance of small diffusible molecules from dermis, particularly the upper dermis, is highly dependent on the local blood flow [22]. This is most likely applicable for nicotine uptake in the present study. Heat accelerates skin blood perfusion which affects both nicotine passages through the skin and its diffusion from cutaneous and subcutaneous tissue into the systemic circulation.

We measured the nicotine uptake indirectly by subtracting the residual nicotine in the patch from the initial patch content. Since the initial content in the patches varied to some extent (7%), it could have been desirable to obtain blood samples and measure the nicotine content in plasma. This could confirm that nicotine mainly entered the systemic circulation and was not stored in the dermis or being vaporised. However, blood sampling is an invasive procedure and requires trained personnel.

In the present study, a link between elevated blood flow and higher uptake of nicotine was seen in some of the subjects. Due to small number of subjects and intra individual variations and also the patch content variation, no correlation was found between enhanced skin perfusion and the amount of drug uptake.

Theoretically, if following the application of controlled heat the drug uptake increases, it may improve the therapeutic effect of a given drug at a time when it is required. It is worth mentioning that the drug concentration should remain within the therapeutic range to avoid any toxic side effect. In addition, one should consider that tissue perfusion is not the only rate-limiting factor in the absorption of some drug molecules. The influence of heat on the rate of absorption should be further investigated for different drugs in order to validate controlled local heat application as a method in cutaneous drug delivery. Controlling factors are the time and the level of heat application. Hence, controlled heat could be applicable in drug delivery studies with desirable time course and stimulus parameters that can fit into the purpose of drug delivery.

In conclusion, this study demonstrated that local controlled heat significantly increased skin blood flow which led to a marked increase in the transdermal drug uptake from the nicotine

patch. Further studies are required to investigate whether similar heating paradigm can produce comparable effect in drug uptake from other patches e.g. fentanyl, clonidine or scopolamine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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