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Investigating the potential of non-thermal microwave as a novel skin penetration enhancement method

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ABSTRACT

Microwaves (MW), a part of the electromagnetic spectrum at 0.3–300 GHz, affect human body in different ways through its thermal and athermal effects, including fluidization of cell membranes and liquid crystalline systems. Due to presence of such structures in skin barrier, it was decided here to investigate the potential of athermal MW as skin penetration enhancer. In this investigation, nitrofurazone was chosen as the model penetrant and its permeation through rat skin was studied in vitro at 45 and 90 min exposure intervals using MW intensities of 3, 15, 30, 60, 120 W at 2450 MHz. Results revealed that at 30 °C and 45 min exposure, 3 W MW does not affect permeation of nitrofurazone ($P = 0.148$), while higher intensities increased its flux significantly ($P < 0.05$) in a intensity-dependent manner up to 2.7 times. When the duration of exposure increased to 90 min, the enhancement ratio also increased to reach a maximum of 3.3. Applying 60 W MW at 25, 30, 37 and 42 °C resulted in a parabolic relationship between temperature and enhancement ratio. The present results reveal that microwave can act as a skin penetration enhancement method and that its effect depends on applied intensities, exposure time and temperature.

1. Introduction

One of the main problems in development of transdermal drug delivery systems is high barrier properties of intact skin toward permeation of drugs. To solve this problem, various enhancement methods have been employed, including chemical penetration enhancers (Barry, 2001; Williams and Barry, 2004) and physical enhancement methods like iontophoresis (Naik et al., 2000; Barry, 2001), sonophoresis (Mitragotri et al., 1996) and electroporation (Wang et al., 1998).

There are other physical methods that, due to their properties, can be considered as a potential percutaneous penetration enhancement methods, but have not been studied yet, of which one is microwave radiation (MW); the subject of the present study.

Interaction of electromagnetic fields and various life processes have been attractive for scientists since the 18th century. Microwave radiations are part of the electromagnetic spectrum and considered to be the radiation ranging in frequency from 300 MHz to 300 GHz, which correspond to a wavelength range of 1 m to 1 mm (Banik et al., 2003). MWs are present in our daily environment, increasingly, for example, in microwave oven, satellites, radio/TV transmission, cellular-phone, occupational use, and exposure through diathermy (Nakamura et al., 2003).

It has been shown that exposure to microwave can cause harmful effects on different biological systems including hematopoetic system (Busljeta et al., 2004), cardiovascular system (Braune et al., 1998) and uteroplacental function (Nakamura et al., 2003). In fact, this non-ionizing radiation is absorbed at molecular level and manifests as changes in vibrational energy of the molecules (CSIRO, 1994); this proposed mechanism is probably responsible for some of the above mentioned effects.

As far as permeation of molecules through barriers is concerned, it has been shown that microwave increases permeation of gas through polar polymeric membranes at 25 °C, where microwave increases the mobility of polar functional group such as hydroxyl groups, and then, gas permeability improves because of increasing the free volume and/or temperature of the membrane (Nakai et al., 2002). It has also been reported that microwave can increase permeability of some ions such as Na^+ or Ca^{2+} through cell membranes at body temperature (see CSIRO, 1994).

Microwave implies its biological effect both thermally and non-thermally (Banik et al., 2003). To the best of our knowledge, the effects of microwave radiation on transdermal permeation of drugs have not been studied, but, it has been shown that microwave can increase human skin surface temperature (Walters et al., 2000). Therefore, increased percutaneous absorption of drugs through its thermal effects is expected. However, such a mechanism is not studied well and deserves full exploration. The present study focuses on the importance of the other mechanism, non-thermal or athermal effect, of microwave radiation on percutaneous

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absorption of drugs and the objectives are to investigate the possible non-thermal enhancement effects of microwave on percutaneous absorption and also to determine the effects of microwave intensities, exposure time and environmental temperature on this phenomenon.

2. Materials and methods

2.1. Materials

Nitrofurazone (pharmaceutical grade) was supplied by Ubichem (England), and acetonitril was obtained from Carlo Erba (Italy). Triethanolamine, phosphoric acid (85%, w/w), acetone and chloroform were supplied by Merck (Germany). All materials were used as received without further purification.

2.2. Microwave application

Microwave was applied using a microwave industrial generator (MARS X, CEM Co., USA) operating at frequency of 2450 MHz. The system was able to measure the temperature with a sensor and keep it constant at the desired adjusted temperature using a pulsed microwave delivery method. This method eliminates any possible thermal effects. As the microwave effects depend on field strength and duration of exposure (Banik et al., 2003), in this investigation, microwave was applied to the skin samples in two different pulsed methods of (a) repeated 45 min exposures (on/off = 45 min/90 min) and (b) repeated 90 min exposures (on/off = 90 min/45 min) and at different intensities of 3, 15, 30, 60 and 120 W.

2.3. In vitro permeation studies

The permeation experiments were assessed in home-made Franz-type static diffusion cells (surface area = 4.5 cm^2) using excised abdominal full-thickness skin of a male *Sprauge Dawley* rats (weighted 200–250 g). After sacrificing the animals, the abdominal hair was shaved with electric clipper (Mozer, Germany) and full-thickness abdominal skin was separated surgically. The skin samples were then cleaned from subcutaneous fat, muscle and vasculature and kept frozen at −18 to −20 °C until use, not later than 30 days. It has been shown that storage of human skin at −20 °C even up to 450 days (15 months) does not change its permeability to water (Harrison et al., 1984).

Before each experiment, the skin samples were defrosted at room temperature and mounted between the cell compartments with the stratum corneum facing towards the donor compartment. A mixture of distilled water, acetonitril and triethanolamine (790:200:10) was used as the receptor phase and saturated nitrofurazone solution in distilled water was employed as the donor phase.

For each experiment, skin samples were first equilibrated with donor and receptor media overnight by placing 30 ml receptor medium in the receptor compartment and distilled water in the donor compartment. After this phase, both compartments were emptied and washed with fresh phases. 4 ml drug-containing donor phase was then placed in the donor compartment and the receptor chamber was filled with 30 ml receptor phase and this time was considered as time zero. Serial samples (4 ml) of receptor solution were collected each 45 min and replaced with fresh solution.

To study the effect of temperature, experiments were performed at different temperatures of 25, 30, 37 and 42 °C. These temperatures are chosen based on lipid thermal transition of skin (Moghimi et al., 1997) as explained later.

Nitrofurazone content of samples was determined using UV spectrophotometer (Spectronic 601, Milton-Roy, USA) at 385 nm. Nitrofurazone flux through the skin was calculated by plotting the cumulative amount of the penetrated drug against time and determining the slope of the linear portion of the graph by linear regression analysis. The effectiveness of microwave is presented here as the "enhancement ratio" (ER) that is calculated by dividing the nitrofurazone steady-state flux in the presence of microwave by that of untreated samples (control).

Statistical comparisons was through two-tailed *t*-test analysis of data which was performed using SPSS 15.0 for Windows, Version 2 (SPSS Inc. Copyright 1989–2006).

3. Result and discussion

3.1. Effects of microwave intensity and exposure time on nitrofurazone permeation

Table 1 provides the effects of different intensities of microwave (3–120 W) in "on/off = 45/90" method on the permeation of nitrofurazone through rat skin at 30 °C. Nitrofurazone showed flux and permeability coefficient of 1.9 $\mu g\,cm^{-2}\,h^{-1}$ and 8.0×10^{-3} $cm\,h^{-1}$ respectively in the absence of microwave (control). Applying MW energy at 3 W increased the values marginally by about 1.1 times, but the difference was not statistically significant ($P = 0.148$). However, when the microwave intensity was increased to 15 W and beyond (30, 60, 120 W), it showed significant effects ($P < 0.03$) by improving the permeation flux of nitrofurazone by about 1.3–2.7 times (Table 1). The present data show that athermal microwave can increase percutaneous absorption of nitrofurazone and possibly other penetrants.

In agreement to the present results, Nakai et al. (2002) have reported the enhancement effects of microwave (100, 300 and 500 W) irradiation on gas permeation through cellulose acetate membrane. They showed that, the permeability and diffusion coefficients of CO_2 for cellulose acetate membrane increase by microwave energy. Their results also showed that the enhancement ratio (treated/control values) increases with the microwave energy, again in agreement with the current results on rat skin. By comparing the effects of microwave on diffusion coefficient and other parameters, Nakai and co-workers concluded that the enhancement of gas permeation through cellulose acetate membrane by irradiation of microwave was mainly caused by increasing the diffusion coefficient.

Considering the above mentioned argument and the fact that microwave can increase molecular vibration, transformation and alteration (Porcelli and Cacciapuoti, 1997; Repacholi, 1998; Banik et al., 2003), it is possible that microwave increases permeation of nitrofurazone through rat skin by increasing its diffusion coefficient through the stratum corneum by disruption of its lipid or protein domains, between which and as discussed later based on other data, intercellular lipid fluidization is apparently more involved in this phenomenon.

In order to evaluate the importance of microwave exposure duration in its enhancement effect, the duration of exposure was increased to 90 min by changing the protocol to "on/off = 90 min/45 min", again at intensities of 3, 15, 30, 60 and 120 W. Results (Table 2) showed that enhancement ratios of microwave at 90 min exposure (on/off = 90/45), are higher than those at 45 min exposure (on/off = 45/90) and reached to 3.3 at 120 W. Besides, opposite to "on/off = 45/90", the enhancing effect of "on/off = 90/45" at 3 W showed to be significant ($P = 0.01$) in comparison to control values. These data show that by increasing the exposure time of microwave, it is possible to increase its enhancement effect possibly due to the fact that damage of electromagnetic radiation is cumulative (Hardell and Sage, 2008).

Comparison of the data of the two used protocols (Tables 1 and 2) shows that enhancement ratios of different methods are equal

Table 1
Effects of microwave on permeation of nitrofurazone through rat skin using "on/off = 45 min/90 min" protocol. Data are mean ± SD (n = 4–5) and are compared to control statistically by two-tailed t-test analysis.

Power (W)	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	Permeability coefficient ($\times 10^3\,cm\,h^{-1}$)	Enhancement ratio[a]	P-value
0(control)	1.91 ± 0.31	8.0 ± 1.31	–	–
3	2.09 ± 0.22	8.8 ± 0.92	1.1	0.148
15	2.38 ± 0.55	10.0 ± 2.29	1.3	0.032
30	3.22 ± 0.88	13.5 ± 3.69	1.7	<0.0001
60	4.27 ± 0.74	17.9 ± 3.09	2.4	<0.0001
120	5.05 ± 0.32	21.2 ± 1.34	2.7	<0.0001

[a] Treated/control flux valves.

Table 2
Effect of microwave on permeation of nitrofurazone through rat skin using "on/off = 90 min/45 min" protocol. Data are mean ± SD (n = 4–5) and are compared to control statistically by two-tailed t-test analysis.

Power (W)	Flux($\mu g\,cm^{-2}\,h^{-1}$)	Permeability coefficient ($\times 10^3\,cm\,h^{-1}$)	Enhancement ratio[a]	P-value
0(control)	1.91 ± 0.31	8.0 ± 1.31	–	–
3	2.41 ± 0.36	10.1 ± 1.52	1.3	0.01
15	2.84 ± 0.48	11.9 ± 2.0	1.5	0.001
30	4.58 ± 0.54	19.3 ± 2.28	2.4	<0.001
60	4.99 ± 0.96	21.0 ± 4.04	2.6	<0.001
120	6.34 ± 0.77	26.6 ± 3.22	3.3	<0.001

[a] Treated/control flux values.

Table 3
Comparison of the enhancement effects of microwave at different "on/off" (min/min) regimens but at equal "intensity × time" values.

Power × time (W min)	Enhancement ratio	
	On/off = 90/45	On/off = 45/90
15 × 90 or 30 × 45 = 1350	1.5	1.7
30 × 90 or 60 × 45 = 2700	2.4	2.4
60 × 90 or 120 × 45 = 5400	2.6	2.7

at equal values of "power intensity (W) × exposure time (min)" in the range studied (Table 3). "Power intensity (W) × exposure time" equals the amount of energy provided (e.g. Joule = W s) and these data show that irrespective of the delivery protocol, the amount of delivered energy looks to be crucial and determining in enhancement effect of microwave. This again shows that microwave enhancing effect, at least in the conditions applied here, is cumulative. Such a property would come very helpful in designing and application of the corresponding instruments.

3.2. Effect of temperature on microwave enhancement action

It has been shown that enhancement effect of penetration enhancers is temperature-dependent and mainly happens when the mechanism of action of the enhancer and effects of thermal energy on the barrier are the same, as was shown for effects of cineole toward permeation of 5-fluorouracil through lipid membranes (Moghimi et al., 1997). To investigate this phenomenon for the effects of athermal microwave and based on the main transition temperature (Tm) of skin (35–37 °C), the effects of non-thermal microwave on the permeation of nitrofurazone was studied at four different temperatures of 25 and 30 °C (below Tm), 37 °C (at Tm) and 42 °C (above Tm) here.

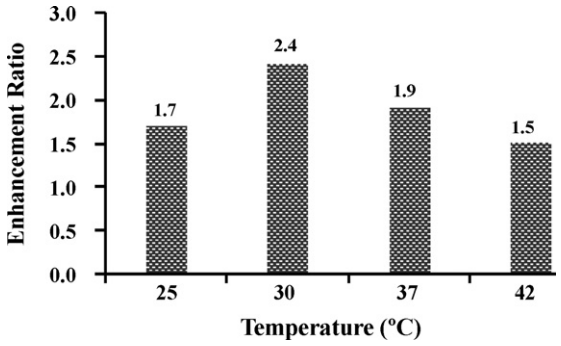

Fig. 1. Effects of temperature on enhancement effect of microwave (60 W, on/off = 45 min/90 min) toward permeation of nitrofurazone through rat skin, indicating a parabolic relationship between temperature and enhancement ratio.

Results (Table 4) showed that while in both cases, absence and presence of microwave, permeability coefficient of the penetrant through rat skin tends to increase by increase in temperature, microwave enhancement ratios show a parabolic relationship with temperature; increase upto 30 °C and decrease at higher temperatures (Table 4 and Fig. 1).

This might show that both systems (athermal microwave and heat) obey a same mechanism or share same sites of action and, therefore, when the barrier properties of skin is reduced by temperature, no room is left for microwave effect. Such a parabolic relationship is reported by Moghimi et al. (1997) when two enhancement methods (thermal energy and chemical enhancers) were applied for enhancement of permeation of 5-fluorouracil through a lamellar liquid crystalline structure as a model for human stratum corneum intercellular lipids.

Table 4
Effects of temperature on enhancement effect of microwave (60 W, on/off = 45 min/90 min) toward permeation of nitrofurazone through rat skin. Data are mean ± SD (n = 4–5) and are compared statistically by two-tailed t-test analysis.

Temperature (°C)	Permeability coefficient ($\times 10^3\,cm\,h^{-1}$)		Enhancement ratio (microwave/control)	P-value
	Control	Microwave		
25	7.04 ± 1.66	12.5 ± 1.72	1.7	0.002
30	8.0 ± 1.31	17.9 ± 3.09	2.4	<0.001
37	10.6 ± 2.56	20.9 ± 2.37	1.9	<0.001
42	12.1 ± 2.00	18.5 ± 2.97	1.5	<0.001

Using human skin, it has been shown that heating in the range of 25–80 °C affects the lipids only, while protein effects occur at temperatures above 100 °C (Al-Saidan et al., 1998). Therefore, the current enhancement due to increased temperature might be attributed to lipid domain of the skin barrier and, considering the above mentioned parabolic relationship and the corresponding argument, we might conclude that athermal microwave induces its effect mainly thorough disruption of lamellar lipid structure of the stratum corneum. It worths to emphasise that the proposed similarity between location of action of athermal microwave and thermal energy does not necessarily mean production of heat by microwave in the lipid domain, as lipid disruption can occur by different mechanism like molecular motion, solubilization, etc.

4. Conclusion

The results of the present investigation clearly show that microwave increases percutaneous absorption of nitrofurazone and possibly other penetrants through its non-thermal effects, a property that is affected by intensity, exposure time and environmental temperature. Further investigations are required in this area to elucidate all aspects of this enhancement method. This technique might open new horizons in the field of transdermal drug deliver.

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