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Transdermal iontophoretic delivery of timolol maleate in albino rabbits

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Abstract

The use of transdermal iontophoresis is a promising technique for the systemic delivery of water soluble and ionic drugs of relatively large molecular size. The present study investigates the skin pre-treatment with Azone® (laurocapram) and iontophoresis on the pharmacodynamic effect of timolol maleate (TM) in vivo in albino rabbits. The pharmacodynamic effect of TM was evaluated by transdermal delivery and compared with an intravenous (i.v.) administration. Iontophoresis of TM (0.1 mg/ml) produced a significant inhibition in the isoprenaline (ISP)-induced tachycardia. Iontophoresis with higher concentration of TM (1 mg/ml) produced a 100% inhibition of the ISP induced tachycardia. Pre-treatment of skin with Azone® (3% v/v emulsion) eliminated the lag time and prolonged the duration of action of iontophoresis from 4 to 6 h. The AUC of Azone® treated group was significantly higher than that of the untreated group ($P < 0.05$). Further, the AUC with iontophoretic delivery and pre-treatment of Azone® was comparable to that of intravenous TM (30 μg/kg). In conclusion, iontophoresis in combination with Azone® can increase the transdermal delivery of TM, whereby the required transport rate can be achieved with a lower drug concentration.

Keywords: Transdermal; Azone®; Iontophoresis; Timolol maleate; Rabbits

1. Introduction

Transdermal iontophoresis is the administration of ionic therapeutic agents through skin by the application of an electric current. This technique has gained growing acceptance for the topical delivery of drugs. Transdermal iontophoresis is currently used in the topical delivery of local anaesthetics (Russo et al., 1980) and anti-inflammatory agents (Lark and Gangarosa, 1990) as well as in the treatment of hyperhidrosis (Elgart and Fuchs, 1987). This technique has also been used in the diagnosis of cystic fibrosis (Panus and Banga, 1997). Furthermore, iontophoresis of fluoride was proven, in double blind controlled, clinical studies, to be effective for the desensitisa-

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tion of hypersensitive dentin (Gangarosa et al., 1989). A review of literature reveals that transdermal iontophoresis would be a promising alternative to passive diffusion in the systemic delivery of water-soluble, ionic drugs of relatively high molecular weight including peptide drugs. Iontophoresis has been shown to be a safe means of delivering peptides and other molecules in a non-invasive and controllable fashion (Green, 1996). Further, because the amount of drug transported by iontophoresis is proportional to the current applied, it is possible to deliver the drug in a controlled manner using pre-programmed delivery rates (Sage, 1993).

The effect of combining iontophoresis with various chemical penetration enhancers has been studied by a number of researchers (Srinivasan et al., 1990; Bhatia and Singh, 1999). Most of them showed that penetration enhancers increased the iontophoretic transport of drugs (Bhatia and Singh, 1998), though some of the penetration enhancers had no effect or decreased the iontophoretic transport (Wearley and Chien, 1990; Hirvonen et al., 1993). However, the in vivo performance of the combination of penetration enhancers and iontophoresis has not been studied in detail. The aim of the present study was to investigate the effect of combination of iontophoresis and a chemical penetration enhancer on the pharmacodynamic parameters such as lag time, magnitude and duration of action of timolol maleate (TM) in vivo in rabbits. Azone® (laurocapram) is a chemical that was developed specifically as a skin penetration enhancer in order to improve the absorption of drugs through the skin. Azone® has been found to enhance the percutaneous penetration of both hydrophobic and hydrophilic drugs (Kai et al., 1993; Barry and Williams, 1995).

TM is a nonselective beta-adrenergic blocking agent without membrane stabilising or intrinsic sympathomimetic activities. TM is used in the management of hypertension, angina pectoris, myocardial infarction and glaucoma. It undergoes extensive first-pass hepatic metabolism and its elimination half-life is 2–2.6 h. Transdermal delivery of TM would avoid hepatic first pass metabolism after oral administration. In the present study TM has been used as a model drug to study the in vivo performance of transdermal iontophoresis and the effect of a penetration enhancer laurocapram (Azone®) in combination with iontophoresis. Two different concentrations of TM and the effect of pre-treatment of Azone® on the iontophoresis was studied and the suppression of the tachycardia produced by a standard dose of isoprenaline (ISP) was used as the pharmacodynamic parameter. Furthermore, the effect of iontophoretically delivered TM was compared to that of intravenous (i.v.) administration.

2. Materials and methods

2.1. Materials

TM and Azone® were gifts from Merck Sharp Dohme, Hertz, UK and Nelson Research Corp., CA, respectively. All other chemicals were of analytical grade. A constant current source was used and platinum electrodes (99.9% pure) were modified before use at the Science Instrumentation Center, Banaras Hindu University, Varanasi, India. The diffusional surface area of the side-by-side diffusion cell was 3.14 cm^2 and the volume of the half cell was 5 ml. Albino rabbits (1.5–2.0 kg) of both sexes were obtained from Zoological Animals Emporium, Varanasi, India. A Students Physiograph (Bio-Devices, Ambala, India) was used to record the electrocardiogram (ECG).

2.2. Preparation of solution/emulsion

ISP solution (2.5 μg/ml free base) was prepared by dissolving isoprenaline sulphate (Burroughs Wellcome, Bombay, India) in sterile normal saline (0.9%). Heparin solution (500 IU/ml) was prepared from Beparine® (Biological E. Ltd., Patancheru, India) by dilution with sterile normal saline. A 3% v/v emulsion of Azone® in water was prepared using 0.11% v/v of Tween-20 as an emulsifier (Sugibayashi et al., 1985). The Azone® emulsion was prepared by homogenising the above mixture at high speed for 20 min using a laboratory homogeniser (Remi Instruments, Bombay, India).

2.3. Preparation of animals

The iontophoretic delivery of TM in vivo was evaluated by measuring the inhibition of ISP-induced tachycardia in rabbits (Kemken et al., 1991). Previous studies indicate that there is a good correlation observed between the plasma timolol concentration and the β-blockade (Jacqueline et al., 1990). The animals were acclimatized to laboratory conditions (25 ± 1°C) for 7 days before experiment. Rabbits were on standard rabbit chow and water was supplied ad libitum. The animal was anaesthetised using pentobarbitone sodium (35 mg/kg, i.v. with a maintenance dose of 15 mg/kg, as and when needed). ECG electrodes were connected to the subcutaneous tissue on the lower limbs of the animal. Lead II was used for recording the ECG on a physiograph. The chart speed was maintained at 5 mm/s. Heart rate was determined by counting the 'T-waves' of the ECG. A catheter was placed in the marginal ear vein. Heparinized saline (10 IU/ml) prepared from the stock solution of heparin (500 IU/ml) was filled in the catheter to prevent blood clots and to overcome its dead volume.

2.4. Pharmacodynamic effect of intravenously administered TM

Normal heart rate was recorded before administration of ISP. Three intravenous bolus injections of a standard dose of isoprenaline sulphate (equivalent of 0.25 μg/kg of ISP) were administrated at intervals of 20 min. Heart rate was monitored from −2 to 20 min after each ISP administration. TM (10, 20 and 30 μg/kg body weight) was slowly injected intravenously and the effect of ISP was determined every 30 min up to 3 h and then every hour up to 8 h. The difference in heart rate before each ISP administration and the maximum heart rate achieved upon each ISP administration was calculated. All the experiments were performed at least in triplicate.

2.5. Pharmacodynamic effect of TM delivered transdermally by iontophoresis

The hair on one side of the lateral-lumbar region was removed using iris scissors 24 h prior to experiment taking care not to damage the stratum corneum. Two hollow, cylindrical glass chambers with an internal diameter of 2 cm were affixed 2 cm apart on the hair-free skin using adhesive Fevibond® (Green et al., 1992). Phosphate buffer (pH 7.4; 0.2 M) containing TM (0.1 or 1.0 mg/ml; 5 ml) was placed in the anode and 5 ml of fresh phosphate buffer (pH 7.4; 0.2 M) was placed in the cathode chamber. Platinum electrodes were inserted through tight-fitting caps in the anode and cathode chambers. Current at 0.375 mA/cm^2 was applied for 2 h and the drug solution remained in the chamber for up to 8 h. ISP was administered every 30 min up to 3 h and then every hour up to 8 h.

Normal heart rate was recorded before the administration of ISP. ISP (0.25 μg/kg i.v.) was administered thrice at the intervals of 20 min. The heart rate was recorded before and up to 20 min after the administration of ISP. The difference in heart rate before and the maximum heart rate after ISP administration was calculated. In studies with a penetration enhancer, 5 ml of Azone® emulsion (3% v/v with 0.11% v/v Tween-20) was placed in the anode chamber. At the end of the 3 h pre-treatment period, the enhancer solution was thoroughly washed with tri-distilled water. Then 5 ml of TM solution was placed in the anode chamber and iontophoresis was performed as described before. All the experiments were carried out at least in triplicate.

The percent inhibition of ISP-induced tachycardia was calculated by the following formula (Ishizaki and Tawara, 1978)

$$\%\ \text{Inhibition} = \frac{HR_0 - HR}{HR_0} \times 100$$

where, HR_0 is the number of beats increased by ISP before drug administration and HR is the number of beats increased by ISP during and after drug administration.

2.6. Data analysis

The percent inhibition of ISP induced tachycardia was plotted against time and the area under the curve (AUC) was determined by trapezoidal rule. The maximum effect (E_{max}), the time to reach the maximum effect (T_{max}), and the lag time

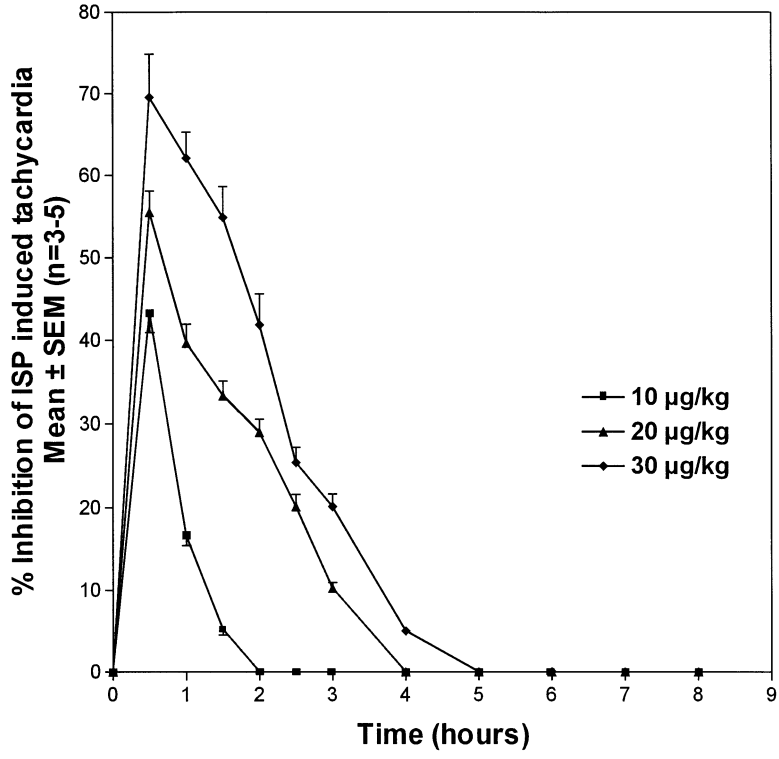

Fig. 1. Effect of intravenous administration of TM (10, 20 and 30 μg/kg) on the ISP induced tachycardia in rabbits.

were determined from the graphs. Student's *t*-test was performed to determine the level of significance. Data was considered significant at $P < 0.05$.

3. Results and discussion

Fig. 1 shows the effect of intravenously administered TM on the inhibition of ISP-induced tachycardia in albino rabbits. The AUC, T_{max} and E_{max} of the intravenously administered TM are presented in Table 1. As the dose of TM increased from 10 to 30 μg/kg, the E_{max} and duration of effect also increased. However, T_{max} was the same for all three doses. A maximum of about 70% inhibition of ISP-induced tachycardia was observed with 30 μg/kg of TM. Furthermore, the results showed a dose dependent increase in the AUC values of TM inhibiting effect ($r = 0.9973$).

Iontophoresis (0.375 mA/cm^2) of TM was performed for 2 h and the pharmacodynamic effect

Table 1
Calculated parameters of in vivo experiments in rabbits[a]

	$AUC_{0-8\ h}$	E_{max}	T_{max}
TM (intravenous)			
10 μg/kg	651.15 (71.65)	43.33	0.5
20 μg/kg	1929.62 (187.82)	55.55	0.5
30 μg/kg	2920.21 (352.18)	69.51	0.5
TM (iontophoresis)			
0.1 mg/ml	2032.00 (169.85)	52.50	2.0
1.0 mg/ml	5637.62 (410.42)	100.00	1.5
TM 0.1 mg/ml (iontophoresis + Azone®)	3400.85 (362.95)	53.04	1.5

[a] E_{max}, maximum effect (% of isoprenaline inhibition); T_{max}, time to reach maximum effect (h); $AUC_{0-8\ h}$, area under the effect time curve during the 8 h experiment (% h), the S.E. values are given in parenthesis.

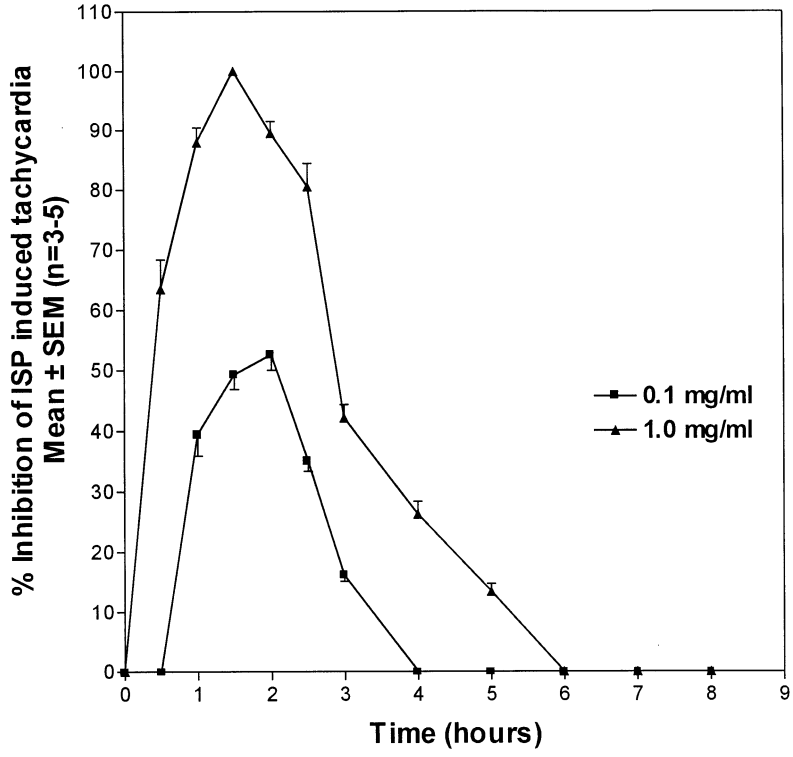

Fig. 2. Effect of iontophoresis of TM (0.375 mA/cm^2 for 2 h) at two different concentrations on the ISP induced tachycardia in rabbits.

was monitored up to 8 h. The effect of iontophoresis with 0.1 and 1.0 mg/ml of TM on the inhibition of ISP-induced tachycardia is presented in Fig. 2 and Table 1. With 0.1 mg/ml of TM in the drug reservoir chamber, a lag time of 0.5 h was observed, and the maximum effect ($52.3 \pm 3.1\%$) was observed at 2 h. With 1 mg/ml, a maximum effect of 100% inhibition of ISP-induced tachycardia was observed at 1.5 h. Unlike at 0.1 mg/ml concentration, there was no lag time observed with 1 mg/ml of TM and the duration of effect was longer (6 h) compared to 0.1 mg/ml, where the duration of effect was 4 h. The AUC value of 1.0 mg/ml of TM was significantly greater ($P < 0.01$) than that of 0.1 mg/ml. Thus, an increase in the concentration of drug from 0.1 to 1.0 mg/ml eliminated the lag time, increased the E_{max}, and reduced the T_{max} (Table 1).

Fig. 3 compares the effect of iontophoretically delivered TM (0.1 and 1.0 mg/ml) with that of intravenously administered TM (30 μg/kg). It can be observed that the onset of action of iontophoretically delivered TM (1.0 mg/ml) was similar to that of intravenous administration. The E_{max} and duration of the effect of iontophoretically delivered TM (1.0 mg/ml) was higher than intravenously administered TM. While the AUC of iontophoretically delivered TM (1.0 mg/ml) was significantly higher ($P < 0.01$) than intravenously administered TM, the AUC obtained from iontophoretically delivered TM (0.1 mg/ml) was significantly lower ($P > 0.05$) than intravenously administered TM.

The transdermal iontophoretic permeation of TM has been reported to increase several fold through human cadaver skin compared to passive diffusion (Soni and Dixit, 1994). The effect of iontophoresis of TM has been recently compared with that of propranolol hydrochloride and sotalol hydrochloride through human cadaver skin

(Hirvonen et al., 1998). The enhancement of permeation by iontophoresis was dependent on the hydrophilicity of the drug. The highest iontophoretic transport was observed with the most hydrophilic drug, sotalol hydrochloride, followed by TM and propranolol hydrochloride.

In an attempt to increase the iontophoretic transport, Azone® 3% v/v was used for pre-treatment of skin for 3 h followed by iontophoresis (0.375 mA/cm^2 for 2 h) with 0.1 mg/ml of TM. Fig. 4 and Table 1 show the results of pre-treatment of Azone® on the iontophoretic delivery of TM in terms of its pharmacodynamic effect in albino rabbits. The data obtained with iontophoresis (0.1 mg/ml, without enhancer treatment) was replotted for comparison. Interestingly, the pre-treatment of skin with Azone® eliminated the lag time and the duration of effect was prolonged up to 6 h compared to the effect of iontophoresis (0.1 mg/ml) without Azone® treatment. The AUC value in the Azone® treated experiment was significantly higher ($P < 0.05$) than in the untreated experiment. However, there was no significant difference in the E_{max}.

Azone® has been reported to enhance the passive transdermal permeation of a wide variety of drugs including timolol (Kai et al., 1993). Azone® increases the transdermal permeation by changing the intercellular structure as characterised by using differential scanning calorimetry (Hirvonen et al., 1994; Barry and Williams, 1995). In the present study, a combination of iontophoresis and a penetration enhancer (Azone®) was studied with respect to changes in the pharmacodynamic effect of TM in vivo in rabbits. Interestingly, pre-treatment with Azone® eliminated the lag time and significantly increased the AUC of the pharmacodynamic effect of TM. Ganga et al. (1996) reported that iontophoresis in the presence of Azone® was more effective in the delivery of

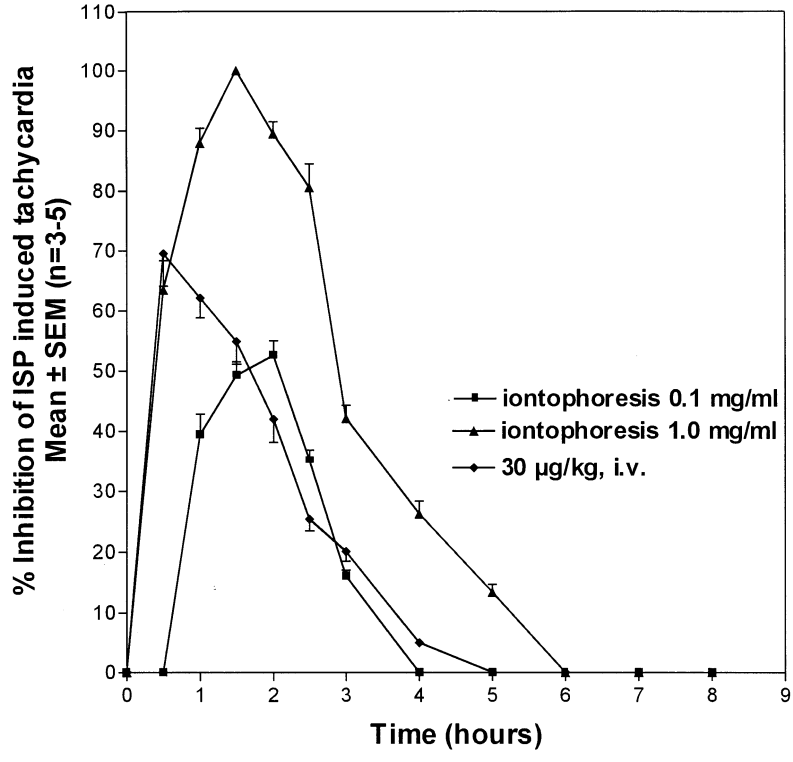

Fig. 3. Comparison of the effect of intravenous administration of TM (30 µg/kg) and iontophoresis of TM (0.375 mA/cm^2 for 2 h) on the ISP induced tachycardia in rabbits.

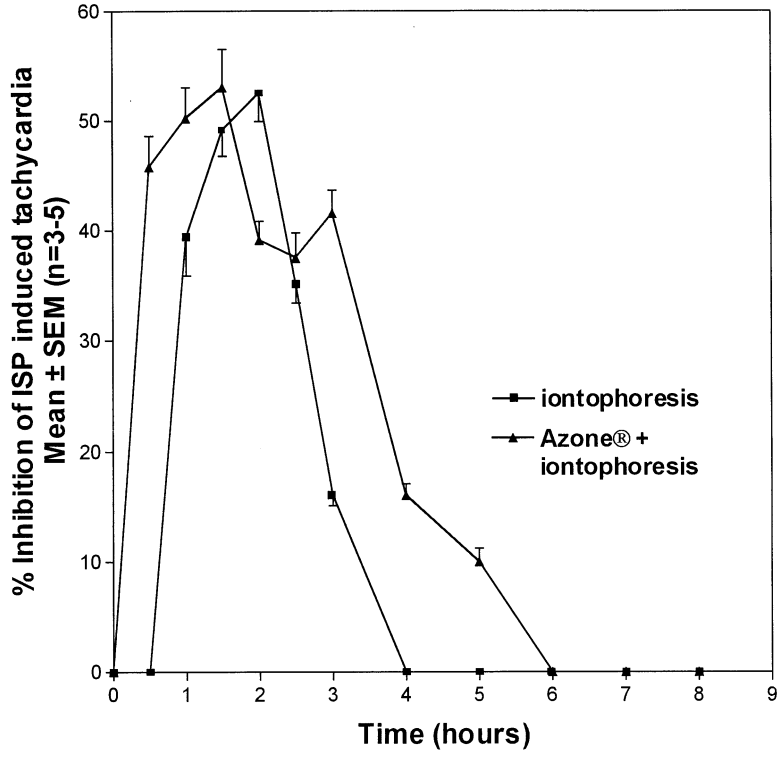

Fig. 4. Effect of pre-treatment of Azone® (3% v/v) for 3 h and iontophoresis of TM (0.1 mg/ml; 0.375 mA/cm^2 for 2 h) on the ISP induced tachycardia in rabbits.

metoprolol tartrate across human skin in vitro. Recently, Kalia and Guy (1997) employed impedance spectroscopy to assess the interaction between Azone® along with other enhancers and the electrical modes of penetration enhancement in human volunteers. Azone® had a profound effect on the post-iontophoretic impedance. Furthermore, the applied electric field might have caused structural reorganisation and enlarged pre-existing channels in the appendageal structures (Kasting, 1992; Kalia and Guy, 1995). The changes in the intercellular structure and reduction in the skin impedance caused by Azone® might be the reasons for the enhancement of TM transport and hence it's pharmacodynamic effect. Thus the present study suggests that the combination of iontophoresis and Azone® emulsion could significantly reduce the concentration of drug required to achieve the desired pharmacodynamic effect. Pre-treatment with Azone® before iontophoresis may also reduce the current levels required to produce the desired effect.

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