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Note

Enhanced skin penetration of gentamicin sulphate by iontophoresis invitro and invivo studies

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Abstract

In the present study enhanced permeation of gentamicin sulphate was achieved across rat skin by use of electric current as compared to passive diffusion. A 65% increase in drug permeation was obtained in the in vitro release studies. In vivo studies showed a manifold increase in serum gentamicin sulphate concentration after iontophoresis. © 1997 Elsevier Science B.V.

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1. Introduction

There has been a growing recognition that the benefits of intravenous drug infusion can be closely duplicated, without its hazards by using intact skin as the port of drug administration to provide continuous transdermal delivery into systemic circulation. iontophoresis, is a process which causes an increased penetration of solute molecules into tissues by the use of an applied current and which has therefore been employed for transdermal delivery of drugs.

Gentamicin is produced by a species of bacteria of the genus Micromonospora and was discovered in Schering Research Laboratories by Weinstein et al. (1963). It is not absorbed after oral administration, and is usually administered by intravenous or intramuscular routes.

2. Materials and methods

For the present study, gentamicin sulphate was obtained from M/s Pharmax Corp Ltd., New

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Table 1
Effect of iontophoretic parameters on cumulative amount of drug released when time of application of current was 15 min

Parameter	Dose (mg/cm^2)	Intensity (mA/cm^2)	Duty cycle	Time (min)	Cumulative amount (mg ± S.D.)	% Enhanced over passive diffusion
Passive	15	—	—	15	0.896 ± 0.031	—
	30	—	—	15	1.220 ± 0.040	—
	45	—	—	15	1.382 ± 0.052	—
Iontophoresis	15	0.5	1:1	15	1.393 ± 0.054	35.68
	30	0.5	1:1	15	1.868 ± 0.081	34.69
	45	0.5	1:1	15	2.004 ± 0.082	31.64
	15	0.75	1:1	15	1.576 ± 0.039	43.15
	30	0.75	1:1	15	2.070 ± 0.065	41.06
	45	1.15	1:1	15	2.165 ± 0.077	36.17
	15	1.15	1:1	15	2.332 ± 0.054	61.58
	30	1.15	1:1	15	2.740 ± 0.089	55.47
	45	1.15	1:1	15	2.937 ± 0.058	53.94

Delhi. Isotonic phosphate buffer was prepared as per USP. The vertical type of diffusion cell was used for in vitro experimentation. The entire assembly is maintained at 37 ± 0.5°C.

For in vivo experiments, bell shaped cell with wide flanges of ground glass at the base was used. The cell was 5 cm in length and had a diameter of 1.5 cm and a capacity of 10 ml. Metal electrodes, made of folded aluminium foil, were made for each iontophoretic experiment.

Rat abdominal skin was employed as the in vitro animal model. For in vitro studies excised skin was soaked in 0.32N NH_4OH isosmotic (pH 10) for 20 min. The separation was completed and the hair and fat layer were cleared. The skin was examined, for pin holes.

Stabilization of skin was carried out using buffer in the donor compartment and stirring for 3 h with a teflon bead. After the preparation and stabilization of skin, the receiver chamber was loaded with fresh IPB (pH 7.4) and the donor chamber was filled with solutions of gentamicin sulphate in distilled water at appropriate concentrations.

For iontophoresis, current was applied at different intensities 0.5, 0.75 and 1.15 mA/cm^2 at 26 kHz 1:1 duty cycle for 15 and 30 min. Anodal current was applied to the drug chamber and cathodal current to the receiver chamber.

Samples were withdrawn from the receiver after every 30 min for 6 h. The temperature was maintained at 37 ± 0.5°C and the receiver chamber was stirred continuously (Nanda and Khar, 1994) on a magnetic stirrer.

For quantitation of gentamicin sulphate, suitable volumes of test solutions were diluted with distilled water. To these dilutions, 1.5 ml of isopropanol was added and mixed. One ml of O-phthalyldialdehyde reagent (USP) was added and mixed. The final solution was allowed to stand at room temperature for 45 min and then scanned for absorption maxima (λ_{max}). This λ_{max} was used for estimation of gentamicin sulphate permeated in the receiver chamber (Sampath and Robinson, 1990). The absorbance values were read against a linear standard plot to obtain corresponding concentrations of gentamicin sulphate.

The results of in vitro experimentation were calculated as the cumulative amount of drug released in mg/cm^2 of skin surface against time and permeation flux (mg/cm^2 per h) against time (Tables 1 and 2).

In vivo pharmacokinetic studies were conducted on male haired albino rats, using the bell shaped diffusion cell. The surface area of skin exposed to the drug penetration was same as for in vitro experiments (Table 3).

For passive/iontophoretic diffusion experiments, the animals were anaesthetized with i.p.

Table 2
Effect of iontophoretic parameters on cumulative amount of drug released in-vitro when the duration of application of current was 30 min

Parameter	Dose (mg/cm^2)	Intensity (mA/cm^2)	Duty cycle	Time (min)	Cumulative amount (mg ± S.D.)	% Enhanced over passive diffusion
Passive	15	–	—	30	0.896 ± 0.030	—
	30	–	—	30	1.220 ± 0.040	—
	45	–	—	30	1.352 ± 0.052	—
Iontophoresis	15	0.5	1:1	30	1.782 ± 0.038	49.72
	30	0.5	1:1	30	2.559 ± 0.031	52.33
	45	0.5	1:1	30	2.627 ± 0.034	47.39
	15	0.75	1:1	30	2.538 ± 0.043	64.70
	30	0.75	1:1	30	2.794 ± 0.077	56.34
	45	1.15	1:1	30	2.879 ± 0.060	52.00
	15	1.15	1:1	30	2.704 ± 0.047	66.86
	30	1.15	1:1	30	2.124 ± 0.089	64.37
	45	1.15	1:1	30	2.243 ± 0.102	58.31

pentobarbitone sodium during the period of drug application (30 min). For passive diffusion 10 ml of 15 mg/ml drug solution in distilled water was loaded in the cell and no current was applied, the cell was emptied after 30 min.

For iontophoretic studies, a similar setup was used with anode dipped in drug solution and the cathode strapped on lower right leg. Current was applied at 5 mA, 20 kHz, 1:1 duty cycle for 30 min. Blood samples were collected at 30, 90, 180 and 360 min—via intra ocular route, analyzed by fluorescence spectrophotometry after suitable preparation.

For calculation of various pharmacokinetic parameters of gentamicin sulphate in rats the elimination was assumed to be of first order. The parameters calculated include K, the elimination rate constant, $t_{1/2}$, the biological half live, V_d, the volume to distribution, and d, the blood clearance.

Table 3
In-vivo pharmacokinetic studies for iontophoretic and passive delivery of gentamicin sulphate

Time (h)	Serum concentration of gentamicin sulphate (ng/ml)	
	Iontophorteic delivery	Passive delivery
0.5	4908 ± 121	472 ± 11
1.5	4661 ± 76	485 ± 26
3.0	3925 ± 107	510 ± 13
6.0	3817 ± 89	469 ± 09

$n = 5$; $k = 0.894\ h^{-1}$; $t_{1/2} = 7.74$ h; $V_d = 6.82$ l; $d = 0.6097$ l/h; AUC = 24 598 ng:h/ml.

3. Results and discussion

Based on the in vitro and in vivo experiments some inferences can be made for the iontophoretic delivery gentamicin sulphate.

Iontophoresis gentamicin sulphate cause an approximately 60% increase in amount of drug delivered across the skin as compared with passive diffusion.

Increasing the amount of drug load in donor cell results in increased amounts of drug delivered across the skin. However, this increase is not directly proportional to the amount increased.

Increasing the intensity of application of current from 0.5 to 1.15 mA/cm^2 results in increased delivery of gentamicin sulphate. At an applied concentration of 15 mg/cm^2, the amount delivered in 6 h increased from 1.393 mg/cm^2 to 2.332 mg/cm^2. At 30 mg/cm^2 the amount delivered increased from 1.868 to 2.332 mg/cm^2 and at 45 mg/cm^2 from 2.004 to 2.937 mg/cm^2.

Increasing the duration of application of current from 15 to 30 min at similar drug loads results in higher volumes of the drug delivered into the receiver chamber. At 15 mg/cm^2 concentration, amount delivered across the skin increased from 1.393 to 1.782 mg/cm^2 at an applied current of 0.5 mA/cm^2. At same applied current, at a drug load of 30 mg/cm^2 the amount delivered increased from 1.868 to 2.559 mg/cm^2 and at 45 mg/cm^2 it increased from 2.004 to 2.627 mg/cm^2.

Passive diffusion of gentamicin sulphate in rats did not establish significant drug levels in blood. However, significant concentrations of gentamicin sulphate were detected in the serum following iontophoretic delivery. After 0.5 h of iontophoresis 4908 ng/ml of gentamicin was detected in serum.

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