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In vivo studies on nasal preparations of ciprofloxacin hydrochloride

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Gel formulations of ciprofloxacin hydrochloride (CPH) were prepared with bioadhesive polymers such as hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC) and methylcellulose (MC). They were administered into the nasal cavity of rabbits. A nasal aqueous suspension of CPH with glycerol was also applied. In addition, the effect of Tween 80 as penetration enhancer was examined. The agar plate diffusion technique was applied for the assay of CPH. The results were compared with oral and intravenous administrations. The bioavailability of the CPH gel formulation prepared with HPMC was almost identical to that of the oral route. Other nasal formulations with HEC and MC had bioavailabilities lower than oral preparations. The relative bioavailabilities for the formulation containing HEC and MC were 48.7 and 45.54%, respectively. To increase the bioavailabilities, 1% (w/w) of Tween 80 was added. The bioavailability of these gel formulations increased to 63.54 and 55.72%, respectively. Experiments carried out on rabbits showed that the nasal administration of CPH bioadhesive gel formulation containing HPMC may be an alternative to the oral route.

1. Introduction

Ciprofloxacin hydrochloride (CPH) is a synthetic quinolone that acts as a specific inhibitor of bacterial DNA-gyrase. It is currently available in tablet, parenteral infusion and ophthalmic formulations. CPH has a plasma half-life of 3–5 h and its recommended dosage is 250 mg or 500 mg 2 times daily [1, 2]. Ocular, nasal, transdermal and topical formulations of CPH have been investigated [3–6]. Studies on nasal absorption of drugs have shown that intranasal drug delivery is a promising way to obtain high bioavailability [7, 8]. Drugs are absorbed rapidly from the nasal cavity, due to the highly vascularized nasal tissue and the relatively leaky epithelium [9, 10]. The nasal delivery of some antibiotics such as sulbenicillin, cephazolin, cephacetrile, gentamicin, roxithromycin and mupirocin has been studied [11–15]. The systemic nasal absorption of CPH has been investigated using the in situ nasal perfusion technique [4]. Various bioadhesive synthetic polymers, e.g. hydroxypropyl methylcellulose (HPMC), hydroxyethyl cellulose (HEC) and methylcellulose (MC), are used in nasal formulations [16].

In our study, the nasal absorption of CPH from different gel formulations prepared with bioadhesive polymers such as HPMC, HEC and MC was investigated. In addition, the effect of Tween 80, frequently used as penetration enhancer [17] was examined. The bioavailability of a nasal formulation containing a viscous and non-polymeric agent (glycerol) was also assessed.

2. Investigations, results and discussion

The calibration curve of CPH was linear in the concentration range 0.5–13.0 μg/ml. The equation for the standard curve was found to be $y = 3.032 \log x + 4.292$ ($r^2 = 0.998$) [x = concentration (μg/ml), y = diffusion distance (mm)]. The assay limit of quantification was 0.5 μg/ml. The accuracy of the assay (assesed as the percentage ratio of the experimentally determined to the actual drug concentration) was 100.78 ± 2.25% at concentration 4 μg/ml (n = 6). The confidence interval of the assay was found to be 98.20–103.36% (p = 0.05). The coefficient of variations (C.V.) for intra- and inter-day presicion at the concentrations 2.0, 4.0 and 9.0 μg/ml were all <4% (n = 9).

The mean serum concentration-time curve of CPH after nasal, intravenous and oral administrations to five rabbits is shown in the Fig. The mean times to reach the peak level (t_{max}) after oral and nasal administrations (with HPMC, HEC, MC) were 1.2 ± 0.4 h and 0.5 ± 0.0 h, respectively. The maximum serum concentration (C_{max}) for oral administration was 3.50 ± 1.97 μg/ml, whereas the C_{max} values for nasal administrations with gel formulations were within the range of 1.05 ± 0.49–1.82 ± 0.34 μg/ml. The pharmacokinetic parameters and the values of absolute and relative bioavailabilities of six different nasal preparations of CPH are summarized in the Table. The mean $AUC_{(0-\infty)}$ value after the intravenous injection of CPH averaged 8.12 ± 2.60 μg · h/ml. After

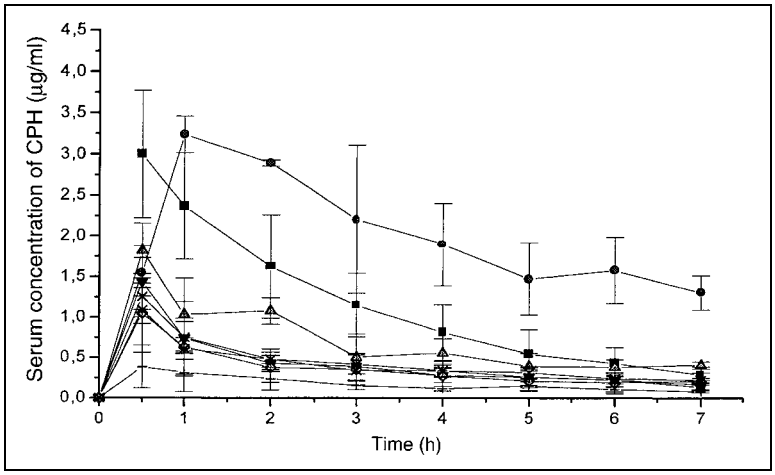

Fig.:
Mean blood levels of Ciprofloxacin hydrochloride in rabbits following intravenous (1), oral (2), nasal-HPMC (3), nasal-HEC (4), nasal-MC (5), nasal-suspension (6), nasal-HEC with Tween 80 (7), and nasal-MC with Tween 80 (8). Points are mean values of 5 animals
—■— 1; —●— 2; —▲— 3; —▼— 4; —◆— 5; —+— 6; —×— 7; —✳— 8

Table: Pharmacokinetic parameters after intravenous, oral and nasal administration of ciprofloxacin hydrochloride in rabbits

Dose (mg)	Route	t_{max} (h)[1]	$t_{1/2}$ (h)[1]	C_{max} (μg/ml)[1]	$AUC_{0-\infty}$ (μg. h/ml)[1]	AB[2](%) ± C.I.[3]	RB[4](%) ± C.I.	CL/F (L/h)
50	Intravenous	0.5 ± 0.0	1.88 ± 0.40	2.99 ± 0.77	8.12 ± 2.60	100	–	6.67 ± 2.03
291	Oral (Tablet)	1.2 ± 0.4	5.79 ± 1.92	3.50 ± 1.97	18.89 ± 3.88	39.97 ± 9.42	100	15.93 ± 3.26
100	Nasal (HPMC)	0.5 ± 0.0	2.80 ± 0.40	1.82 ± 0.34	6.53 ± 0.91	40.21 ± 6.40	100.59 ± 16.02	15.54 ± 2.14
100	Nasal (HEC)	0.5 ± 0.0	2.47 ± 0.38	1.44 ± 0.84	3.16 ± 0.38	19.46 ± 2.71	48.70 ± 6.80	31.99 ± 3.56
100	Nasal (MC)	0.5 ± 0.0	2.64 ± 0.17	1.05 ± 0.49	2.96 ± 0.68	18.20 ± 4.82	45.54 ± 12.09	37.49 ± 8.72
100	Nasal (HEC + Tween 80)	0.5 ± 0.0	3.14 ± 0.38	1.09 ± 0.17	4.12 ± 0.31	25.39 ± 2.19	63.54 ± 5.51	24.36 ± 1.93
100	Nasal (MC + Tween 80)	0.5 ± 0.0	2.82 ± 0.51	1.09 ± 0.17	3.62 ± 0.86	22.27 ± 5.54	55.72 ± 13.87	30.59 ± 5.34
100	Nasal (Glycerin)	0.6 ± 0.2	4.58 ± 0.65	0.40 ± 0.27	1.74 ± 0.43	10.74 ± 3.01	26.88 ± 7.54	53.29 ± 7.69

[1] Each value represents the mean ± s.d. of 5 rabbits
[2] Absolute bioavailability
[3] Confidence Interval
[4] Relative bioavailability calculated by comparing $AUC_{(0-\infty)}$ values (nasal) to $AUC_{(0-\infty)}$ (oral)

the nasal administration with HPMC the total AUC, corrected for the dose administered, was found to be 6.53 ± 0.91 μg · h/ml. The mean $AUC_{(0-\infty)}$ values after nasal administrations of CPH prepared with HEC and MC were found to be lower than that of HPMC. The terminal elimination half-life ($t_{1/2}$) value for oral administration was 5.79 ± 1.92 h, whereas $t_{1/2}$ values for nasal administrations with gel formulations were in the range of 2.47 ± 0.38–3.14 ± 0.38 h. The oral clearance (CL/F) value of nasal formulations containing HPMC was found to be similar to that of the oral administration. CL/F values for other nasal formulations, however, were significantly higher than the CL/F values for oral and HPMC gel formulation ($p < 0.05$). The absolute bioavailabilities of CPH in the nasal formulation containing HPMC and oral tablet were found 40.22 and 39.97%, respectively. There was no significant difference between these values ($p > 0.05$). The nasal formulations with HEC and MC had lower bioavailabilities in comparison with oral administration and the difference was found statistically significant ($p < 0.05$). The relative bioavailabilities of the nasal formulations with HPMC, HEC, MC, and the suspension of CPH with glycerol were determined as 100.61, 48.70, 45.54 and 26.88%, respectively. Tween 80 was used at 1% (w/w) concentration in the CPH gel formulations containing HEC and MC increase the relative bioavailabilities. As expected, the relative bioavailabilities of CPH gels containing HEC and MC were increased to 63.54 and 55.72%, respectively. When the results of the gel formulations were compared to those obtained after nasal application of the suspension formulation with glycerol, it was shown that all gel formulations of CPH had a bioadhesive effect. The CPH nasal formulation containing HPMC was found as effective as its oral formulation. In addition, nasal formulations have the advantage of a faster attainment of the C_{max} value ($t_{max\ (nasal)}$ 0.5 ± 0.0 h vs. $t_{max\ (oral)}$ 1.2 ± 0.4 h). Experiments performed on rabbits showed that nasal administration of CPH bioadhesive gel formulations containing HPMC may be an alternative to the oral route.

3. Experimental

3.1. Materials

CPH was purchased from Medefarma Est., (Switzerland); hydroxypropyl methylcellulose (Methocel K15M) from Colorcon (GB-Orpington), hydroxyethyl cellulose (Natrosol 250 HHX-Pharm.) from Aqualon (D-Düsseldorf); methylcellulose (Methocel A4C Premium EP) from Colorcon (GB-Orpington), glycerol USP, Tween 80, absolute alcohol and lactic acid from Merck (D-Darmstadt), bovine serum albumin from Sigma (Louis), Iso-Sensitest Agar from Oxoid (Hampshire).

3.2. Preparation of Ciprofloxacin hydrochloride gels and i.v. formulation

The gels were prepared after the polymers were presoaked in distilled water overnight at room temperature. The concentrations of HPMC, HEC and MC in the gels were chosen as 1% (w/v), 0.5% (w/v) and 1% (w/v), respectively. Thus, viscosities of the aqueous polymer solutions at 20 °C were 500 cps, 400 cps and 395 cps, respectively. CPH was suspended in the gels at the concentration of 250 mg/ml. For evaluating the effect of penetration enhancer, 1% (w/w) of Tween 80 was added to CPH gels with HEC and MC. A suspension of CPH at the same concentration was prepared with glycerol. For the preparation of CPH i.v. formulation, 500 mg CPH were dissolved in 1 ml absolute alcohol, 1 ml lactic acid and 3 ml distilled water and adjusted to 10 ml with distilled water [18]. The solution was filtered through a membrane filter (Sartorius Minisart® NML) and sterilized in autoclave at 110 ± 2 °C for 30 min.

3.3. Animal studies

Albino rabbits of both sexes weighing approximately 3–3.5 kg were used. They were fed with commercial feed and had free access to tap water. Animals were fasted for approximately 18 h before the day of experiment. They were divided in eight groups of five animals each. Six of the groups were given the nasal preparations, one group the i.v. preparation and one the commercial tablet of CPH. No anesthesia was applied to the animals during nasal and i.v. drug administrations. 0.4 ml of gel preparations or glycerol suspension containing 250 mg/ml of CPH were administered into the nasal cavity of rabbits by applying 0.2 ml to each nostril via a pipette. The commercial tablet containing 291 mg CPH was delivered under aether anesthesia to the esophagus of the rabbit through a cut-end plastic syringe placed into the mouth. The i.v. solution (1 ml) was injected into an ear vein of the rabbits. Blood samples were withdrawn by venipuncture from the opposite ear of the rabbits before and 0.5, 1, 2, 3, 4, 5, 6 and 7 h after drug administration. The blood samples were collected as 1.5 ml in sealed Eppendorf tubes. The sera were separated by centrifugation at 15000 rpm for 10 min. The amounts of CPH in the sera samples were determined by means of the microbiological method.

3.4. Ciprofloxacin microbiological assay

The agar plate diffusion technique was applied for the assay of CPH. *Escherichia coli* ATCC 8739 was used as test organism. From the bacteria suspension, having a transmittance of 25% at 580 nm, 1 ml was dispersed in 100 ml of premelted Iso-Sensitest Agar, then 20 ml of this medium were poured into petri dishes containing reservoirs of 6 mm diameter. Standard solutions in 0.5–13 μg/ml concentration range were prepared by dissolving CPH reference standard in 7% bovine serum albumin. After 50 μl of the standard solution had been applied to the reservoirs, all assay plates were incubated at 37 °C for 24 h. The inhibition zones were measured and a standard curve of CPH was obtained by plotting concentrations versus inhibition zones on semi-log paper. Six experiments were done for each concentration and the equation of CPH calibration curve and determination coefficient (r^2) was calculated. The serum samples taken at 0–7 h were stored at −20 °C until analysis. 50 μl of each serum were applied in triplicate to the reservoirs.

3.5. Pharmacokinetic analysis

The maximum serum concentration of CPH (C_{max}) and the time to reach this concentration (t_{max}) were determined from the individual serum concentration-time profiles after nasal, intravenous and oral administrations. Least-squares regression analysis was employed on the elimination rate constant (k_{el}) and the terminal elimination half-life ($t_{1/2}$) was computed as $0.693/k_{el}$. The oral clearance (CL/F) was calculated from the formula dose/AUC. The absolute bioavailabilities were calculated by comparing $AUC_{(0-\infty)}$ values (nasal and oral) to $AUC_{(0-\infty)}$ (i.v.). The relative bioavailabilities were calculated by comparing $AUC_{(0-\infty)}$ values (nasal) to $AUC_{(0-\infty)}$ (oral). The area under the serum concentration-time curve ($AUC_{(0-7h)}$) was calculated using the trapezoidal rule and $AUC_{(0-\infty)}$ was calculated using the following equations [19]:

$AUC_{(7h-\infty)} = C_{(7h)} / k_{el}$

$AUC_{(0-\infty)} = AUC_{(0-7h)} + AUC_{(7h-\infty)}$

The relationships between the pharmacokinetic parameters and the doses were examined by one-way analysis of variance (ANOVA); the Kruskal-Wallis test [20] was used to evaluate the significance of differences between pairs of data. $P < 0.05$ was considered to be indicative of significance.

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