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Synthesis and Preformulation studies of a Prodrug of Lomefloxacin

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A prodrug of lomefloxacin was synthesized by reacting the free base with aqueous formaldehyde solution in a 1:1 mixture of methylene chloride and methanol. The colourless, crystalline N-hydroxy methyl derivative, m.p. 220° showed a two-fold increase in solubility and a three fold increase in the dissolution rate over the parent drug. The degradation kinetics, pH-rate profile, antimicrobial activity, LD_{50} and physical characteristics such as particle size distribution, angle of repose of the prodrug were studied. The prodrug showed increased solubility and dissolution and gets easily converted to parent drug.

THE prodrug approach can be considered as a transient chemical cover to alter or eliminate undesirable properties of the parent drug molecule. Prodrugs are designed to overcome pharmaceutical and pharmacokinetic problems associated with the parent drug molecule that could otherwise limit the clinical usefulness of the drug[1]. Lomefloxacin (LMX) as hydrochloride a widely used fluoroquinolone antibacterial drug is well absorbed and peak levels in plasma have been reported as 3-5.2 mg/L and bioavailability is >95% after a single 400 mg oral dose[2,3]. In our continuing search for prodrugs of fluoroquinolones[4-6], N-hydroxymethyl derivative of LMX was prepared in order to obtain a prodrug with increased solubility and dissolution rate.

EXPERIMENTAL

Instrumentation and Materials

Ultraviolet spectral measurements were performed with a Bausch and Lomb Spectronic 21 and Beckman DU-64 double beam spectrophotometer. IR spectra were taken using the KBr disc technique on a 5-DX Niclolet machine. ^{1}HNMR spectra were obtained using Bruker 300 MHz or Jeol FX 100 MHz spectrometer. The mass spectra were recorded on Jeol JMS D-300 mass spectrometer. A Perkin Elmer 240-C elemental analyzer was used for C, H, N, analysis. Lomefloxacin hydrochloride was a gift sample from Systopic laboratories. Other chemicals were of reagent grade.

Synthesis of N-hydroxymethyl LMX

LMX free base was first obtained by adding 1N NaOH dropwise to a solution of LMX. HCl in dichloromethane : methanol (1:1) till alkaline to litmus, and stirring at 30° for two hours. The free base was filtered and recrystallized from dichloromethane : methanol (1:1) and had a m.p. of 240° (Reported 239-240.5°)[7]. The N-hydroxymethyl derivative of LMX was synthesized by taking 0.351 g (0.001 mole) of LMX in 50 ml of solvent (dichloromethane : methanol 1:1) with 0.25 ml (0.003 mole) of aqueous formaldehyde solution (37% w/v). The mixture was stirred at 30° using a magnetic stirrer. The solvent was removed by vaccum evaporation and the derivative was recrystallised from a mixture of dichloromethane : methanol (1:1) Yield 79.31%, m.p. = 220°.

$C_{18}H_{21}O_4N_3F_2$ requires C, 56.70; H, 5.51; N, 11.02; found C, 56.52; H, 5.82; N, 10.90. 1R (KBr) broad band at 3260 (OH) and 3100 (COOH) and strong bond at 1725 (C=0) cm^1.

^{1}HNMR (in $CDCl_3$) δ 8.58 (1H, s, H-2) 7.88, (1H, s, H-5), 4.65 (1H, d, J = 11.0) Hz (H-4 a-1), 4.58 (1H, d, J = 11.0 Hz, H-4 a-2), 4.40 (2H, q, J = 7.0 Hz, 1a), 4.09 (2H, br, s 5), 3.37 (4H, m, 2, 6), 3.06 (1H, m, 3), 1.56 (3H, t, J = 7.0 Hz 1b), 1.17 (3H, d, J = 6.0 Hz, 3 a). Mass spectrum m/z (rel. int.) 381 (M+) n.o., 350 $(M\text{-}CH_2OH)^+$(29.0), 295 [350-NH CH (CH_3) $CH_2]^+$ (100), 251 [295-CO_2]+ (59.8), 210 (5.6), 194 (7.0), 179 (8.0), 131 (9.5), 7.0 (13.7).

Preformulation Studies

Aqueous Solubility :

The solubility of the prodrug was compared with LMX free base by shaking 100 mg with 50 ml water over a period of 48 h. at 25±1°. Aliquots were withdrawn at various time intervals and absorbance was read at 280 nm (Fig.1).

Intrinsic Dissolution Rate :

Discs (13 mm in diameter) of N-hydroxymethyl LMX and LMX were prepared by compressing 100 mg of each in a IR hydraulic press at 200 kg/cm² for 15 seconds. The dissolution medium was 500 ml distilled water 25 ± 1° and the paddle speed was 50 rpm. A 5 ml sample was withdrawn at regular time intervals and was replaced by fresh dissolution medium. The absorbance of the sample was read at 280 nm (Fig. 2).

Kinetic Studies and pH-rate Profile :

A UV method was used to study the degradation kinetics of N-hydroxymethyl LMX using a kinetic module attached to Beckman DU-64 spectrophotometer. Absorbance was noted at 220 nm (this being the wavelength at which the absorption of the drug and prodrug differed maximally) at 30 seconds interval for 1 h. at 25°. For pH-rate profile buffer solutions at pH 1.2, 5.8, 6.2, 6.8, 7.4, 8.4 and 9.0 were prepared (phosphate buffer upto pH 6.8 and tris buffer for other pH values) and the rate of decomposition was followed spectrophotometrically (Fig.3).

Particle size of the drug and the prodrug was determined by optical microscopy method. Various diameters were calculated (Table 1). The frequency distribution plot for LMX and N-hydroxymethyl LMX is shown in Fig. 4. The angle of repose of the drug and the derivative was determined by the funnel method.

In vitro antibacterial activity

The antimicrobial activity of the prodrug was compared with that of the drug. Two Gram -ve organisms (*E. coli* NCTC 10418 and *P. aeruginosa*; a clinical isolate) and one Gram +ve organisms (*S. aureus* NCTC 6571) were used in the study. Nutrient agar was used as bacteriological medium. MIC of the prodrug was determined according to the method of Gotto et al[8].

Toxicological Studies

Acute toxicity studies were carried out to determine the LD_{50} value of N-hydroxymethyl LMX and compare it with the reported LD_{50} value of LMX, according to the method of Behrens Kaerber[9].

RESULTS AND DISCUSSION

The condensation of LMX with aqueous formaldehyde yielded N-hydroxymethyl LMX in the following manner;

The t.l.c. examination of the drug and the prodrug (dissolved in dichloromethane : methanol 1:1) on silica gel G plate with developing solvent consisting of a mixture of ethyl acetate, ethanol and dilute ammonia (1:1:2) gave spots with Rf values 0.693 and 0.725 respectively. Introduction

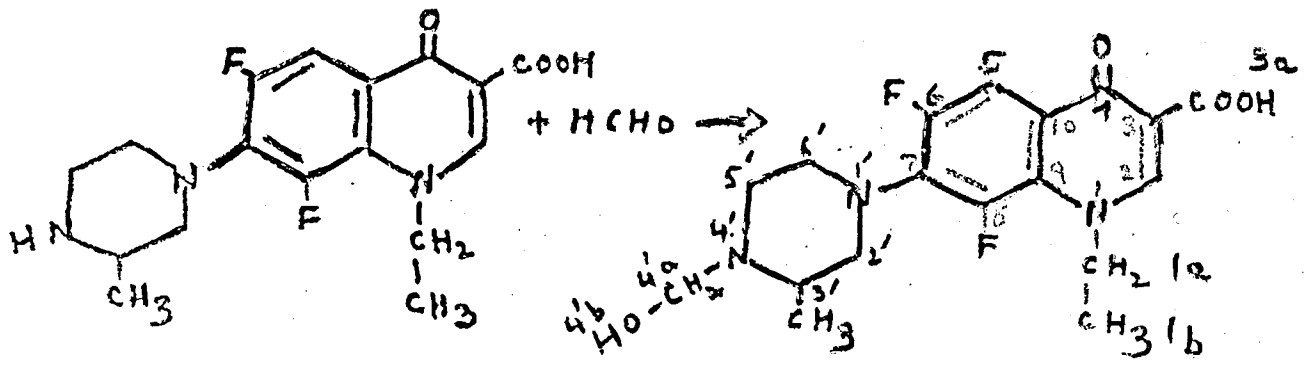

Table 1: Statistical diameters for LMX and N-hydroxymethyl LMX

Diameter	LMX	N-hydroxymethyl LMX
Surface number mean diameter	57.2 μm	54.9 μm
Volume number mean diameter	61.7 μm	59.7μm
Volume surface mean diameter	71.8 μm	70.7 μm
Surface area per unit volume	8.3 x 10^2cm²/cc	8.4 x 10^2cm²/cc

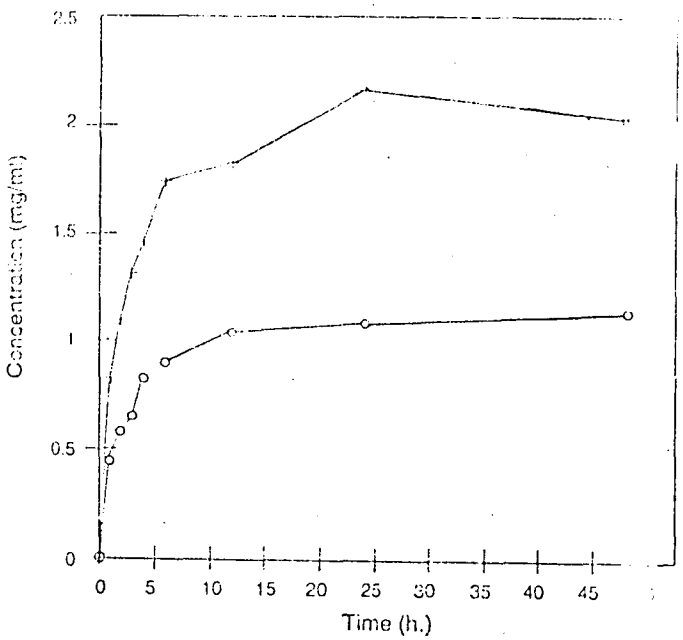

Fig. 1: Solubility Studies of LMX (-o-o-) and N-hydroxymethyl LMX(-+-+-)

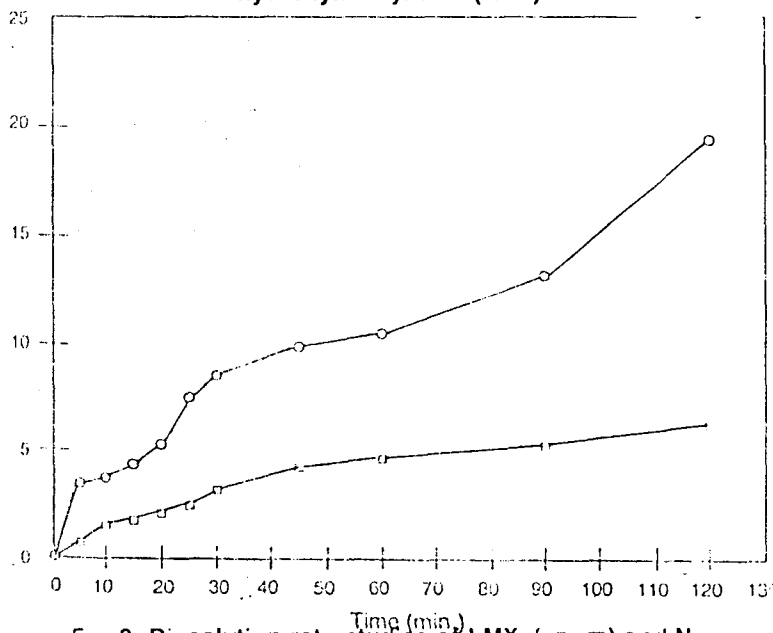

Fig 2: Dissolution rate studies of LMX (-□-□-) and N-hydroxymethyl LMX (-o-o-)

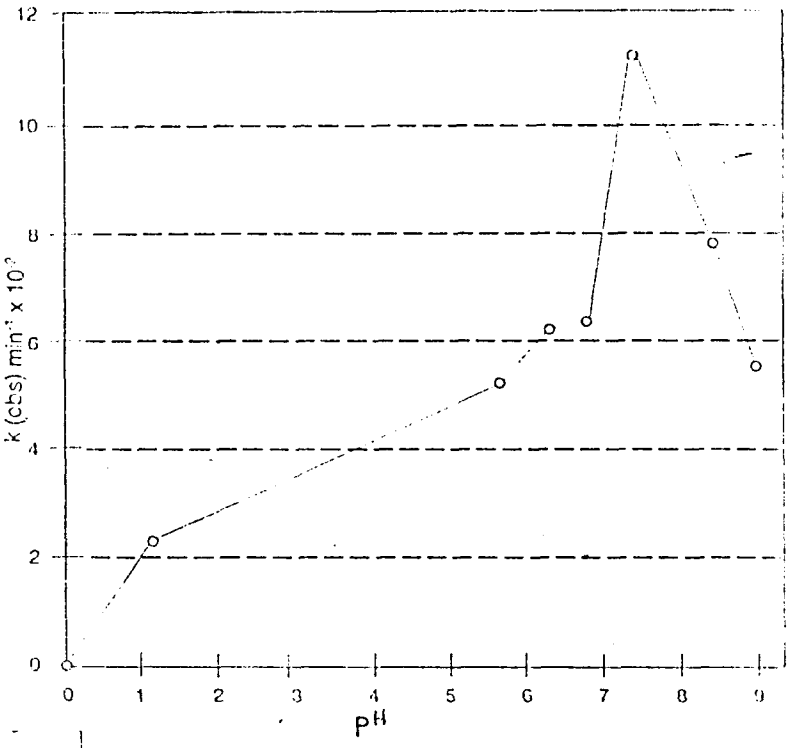

Fig. 3: pH-rate profile of N-hydroxymethyl LMX

of the hydrophillic N-CH_2 OH in the piperazine ring altered the zwitterionic nature of LMX, as the melting point was decreased.

The solubility measurements (Fig.1) indicated an increase in solubility of hydroxymethyl LMX for near y 24 h. after which it decreased due to the conversion of the prodrug to the parent compound. There was a two-fold increase in the solubility of the prodrug (2.13 mg/ml) over the parent compound (1.03 mg/ml)[10]. According to the Noyes-Whitney equation (dc/dt=Ks. Cs), the dissolution rate is directly proportional to the solubility in the diffusion layer. As N-hydroxymethyl LMX exhibited a higher aqueous solubility than the parent drug, it also showed an increase in dissolution rate (Fig.2).

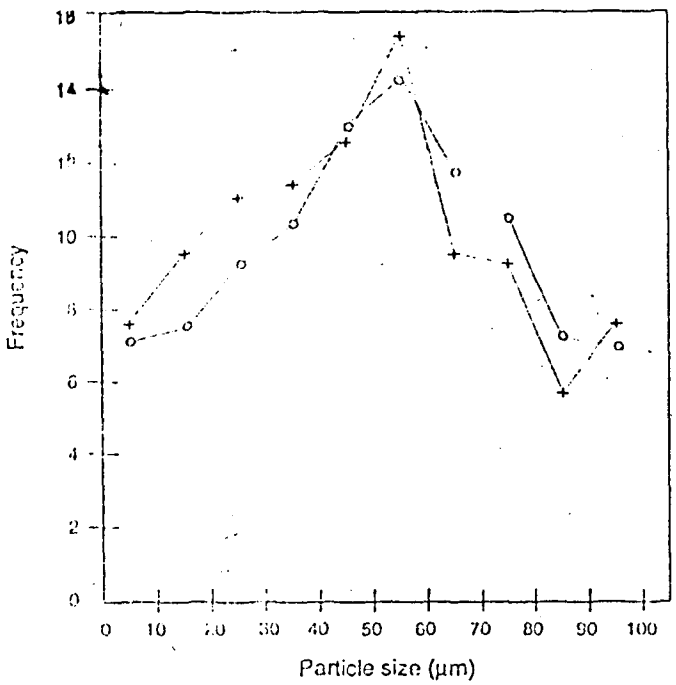

Fig. 4: Frequency distribution plot for LMX (-o-o-) and N-hydroxymethyl LMX (-+-+-)

The degradation of prodrug followed first order kinetics. The N-hydroxymethyl LMX was quite stable at acidic and alkaline pH. Instability at the physiological pH (7.4) ensured prompt conversion of the prodrug to parent drug in plasma (Fig 3). From the particle size determination, various diameters were calculated and are given in Table-1.

Both the drug and the prodrug exhibited low angle of repose 26.5° for LMX and 25.2 for N-hydroxymethyl LMX. This indicates that they may have good flow properties.

The *in vitro* antibacterial activity of the prodrug was found to be similar to the parent drug as equal zones of inhibtion were obtained against all the test organisms studied. This indicated that the condensation of LMX with formaldehyde did not affect the antibacterial activity. The minimum inhibitory concentration of the prodrug was found to be same as that of the parent drug i.e. for S. aureus 1.0 μg/ml[11]. One reason for the same activity could be that due to the unstable nature of N-hydroxymethyl LMX it degraded back to the parent drug during the diffusion time.

In the toxicological study, the LD_{50} value for the derivative was found to be 8.0 mg/kg (Reported value for LMX ≤ 7.8 mg/kg)[12]. This indicates that the substitution of formaldehyde does not have any toxic effect and after decomposition of prodrug, the released formaldehyde also does not show any toxic effect.

It may be concluded that N-hydroxymethyl lomofloxacin has increased solubility and dissolution rate and gets easily converted to the parent drug.

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