RESEARCH PAPER

Received 30 March 1995
Accepted 25 March 1997
Indian J. Pharm. Sci., 1997, 59(3) pp.101-104

Synthesis and Preformulation studies of a Prodrug of Enoxacin

D. CHADHA AND S. P. AGARWAL*
Dept. of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi-110 062.

A prodrug of enoxacin was synthesized by reacting with aqueous formaldehyde solution in a 1:1 mixture of methanol and dichloromethane. The colourless, crystalline N-hydroxymethyl enoxacin, m.p. 262-266° showed enhanced solubility and dissolution rate. The degradation kinetics, pH-rate profile and antibacterial activity of the prodrug were determined.

ENOXACIN is a flourinated 4-quinolone with a broad spectrum of *in-vitro* antibacterial activity and is particularly potent against gram negative organisms and staphylococci. The pharmacokinetic profile of enoxacin is similar to that of ofloxacin achieving higher plasma and tissue concentration and possessing a longer half life than norfloxacin or ciprofloxacin[1].

Enoxacin exists as a zwitterion[2]. In general, for ionizable molecules the rate of transport through biomembranes appears to be proportional to the concentration of undissociated molecules in solution and lipid solubility. Latentiation of the amino function of enoxacin is expected to change the zwitterionic nature and is expected to produce a more readily absorbable form. N- hydroxymethyl derivatives have been reported to exhibit increased aqueous solubility and dissolution rate[3]. Recently, N- hydroxymethyl derivatives of norfloxacin[4] and ciprofloxacin[5] have been reported.

EXPERIMENTAL

Instrumentation and Materials

Ultraviolet spectral measurements were performed with a Bausch and Lomb Spectronic 21 and Beckman DU-64 double beam spectrophotometer using matched 1-cm^2 silica cells. IR spectra were done on a 5-DX Nicolet machine using the KBr disc method. ^{1} HNMR spectra were obtained using a Jeol FX 100 MHz in trifluoroacetic acid (TFA) as solvent. A Perkin-Elmer 240C elemental analyzer was used for C, H, N analysis. An Electrolab tablet dissolution tester TDT-6 was used for dissolution studies. Enoxacin pure drug powder was a gift from Parke Davis & Co. Other chemicals were of reagent grade.

* For correspondence

Synthesis of N-hydroxymethyl enoxacin

N-hydroxymethyl derivative of enoxacin was synthesized by reacting 0.32 g (0.001 mole) of enoxacin in 50 ml solvent (dichloromethane : methanol 1:1) with 0.25 ml (0.003 mole) of aqueous formaldehyde solution (37% w/v). The mixture was stirred for 3 h using a magnetic stirrer. Upon partial removal of solvent by vacuum evaporation, a colourless, crystalline material separated which was recrystallized from a mixture of dichloromethane and methanol. Yield 89%, m.p. 262-266°. $C_{16}H_{19}FN_4O_4.H_2O$ requires C, 52.13; H, 5.75; N, 15.15, found C, 52.43; H, 5.36; N, 15.30 IR (KBr) broad band at 3515 and strong bands at 1765 and 1050 cm^{-1}. ^{1}HNMR (in

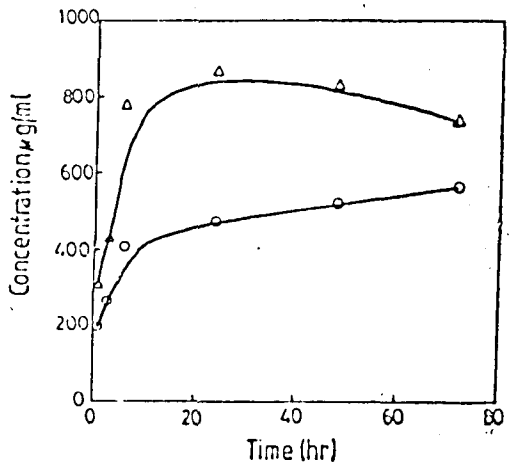


FIG. 1 Solubility Studies

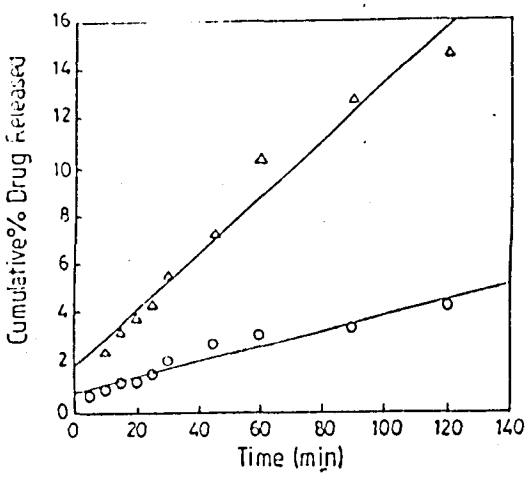


FIG. 2 Dissolution Rate Studies

TFA) δ 8.32 (2H, br. s, H-2, H- 5), 4.86 (2H, br. s, NCH_2 OH), 4.51 (10H, br.s N-CH_2 - CH_3, piperazine 4xCH_2), 1.65 (3H, t, NCH_2 CH_3). The mass spectrum showed) an (M^+-OH) peak at m/z 351 and (M^+-CH_2OH) peak at m/z 337 beside others.

Preformulation studies

Solubility determination

The solubility of N-hydroxymethyl enoxacin was compared with enoxacin by shaking 100 mg of the drug with 50 ml water at 25 ± 1° over a period of 72 h. Aliquots were withdrawn at various time intervals and absorbance was read at 266 nm (Fig. 1).

Dissolution rate

Disc (13 mm diameter) of N-hydroxymethyl enoxacin and enoxacin were prepared by compressing 100 mg of each in a IR hydraulic press at 200 kg/cm^2 for 15 seconds. The dissolution medium was 500 ml distilled water at 25±1° and the paddle speed was 125 rpm. At appropriate time intervals, 5 ml sample was withdrawn and replaced by fresh dissolution medium. The absorbance of the sample was read at 266 nm (Fig. 2).

Kinetic studies and pH-rate profile

A UV spectrophotometric method was used to study the degradation kinetics of N-hydroxymethyl enoxacin using a kinetic module attached to Beckman DU 64 spectrophotometer. Absorbance was noted at 206 nm this being the wavelength at which the absorption of the drug and prodrug differed maximally at various time intervals for 1 h at 25°. For pH-rate profile, buffer solutions at pH 1.2, 4.0, 5.8, 6.2, 6.6, 6.8, 7.4, 8.0, 9.2 and 10.5 were prepared (phosphate buffer upto pH 6.8 and tris buffer for other pHs) and the rate of decomposition followed spectrophotometrically (Fig. 3).

Minimum inhibitory concentration (MIC) and *in-vitro* antibacterial activity

Antibacterial activity of the prodrug was compared with that of enoxacin against two gram positive organisms (*E. Coli* NCTC 10418 and *P. aeruginosa*, a clinical isolate) and one gram negative (*S. aureus* NCTC 6571) using agar plate diffusion method. Mueller- Hinton agar was used as bacteriological medium. The drug was dissolved in isotonic sodium phosphate buffer pH 7.4. MIC of the prodrug was determined according to the method of Gotto *et al*[6].

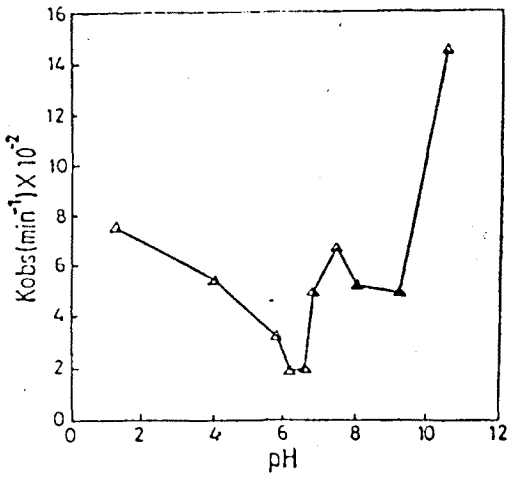
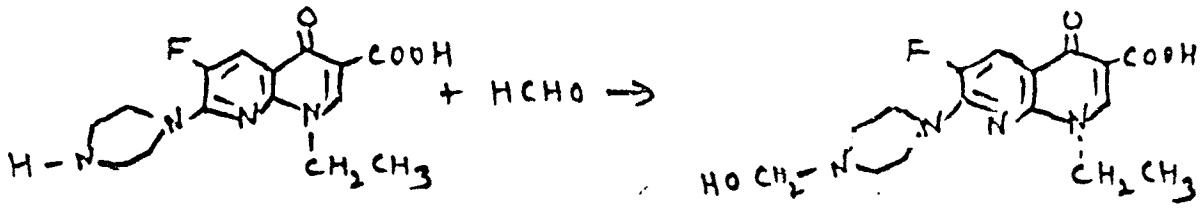


FIG 3 pH rate profile of N-hydroxymethyl ENX

RESULTS AND DISCUSSION

The condensation of enoxacin with aqueous formaldehyde solution yielded N-hydroxymethyl enoxacin according to the following scheme.



The t.l.c. examination of the drug and prodrug (dissolved in methanol: dichloromethane 1:1) on silica gel G plate with a developing solvent consisting of a mixture of ethyl acetate, ethanol and dilute ammonia (1:1:2) gave spots with Rf values 0.69 and 0.73 respectively. The ^{1}HNMR spectral data indicated that the condensation has taken place at the free secondary amino group of the piperazine moiety in enoxacin.

The solubility study (Fig. 1) showed greatly enhanced solubility of the prodrug as compared to enoxacin in the initial stages though as the prodrug got converted to the parent drug, the solubility decreased. The m.p. of the sediment from the prodrug solubility sample was found to be 219-221° (reported m.p. for enoxacin = 220-224°[2,7]). The increase in the solubility of the prodrug may be attributed to the hydrophilic nature of - CH_2OH group. Similarly, the dissolution rate profile (Fig. 2) showed an increased dissolution rate for the prodrug as compared with the parent drug. Dissolution is controlled by the solubility in the diffusion layer and as the N-hydroxymethyl enoxacin is much more soluble in water than enoxacin, it also exhibits increase in dissolution rate.

The decomposition of N-hydroxymethyl enoxacin was found to follow first order kinetics. The prodrug was highly unstable in acidic pH 1.2 ($t_{1/2}$ 9.2 min^{-1}) and in alkaline pH 10.5 ($t_{1/2}$ 4.78 min^{-1}). The pH-rate profile is shown in Fig. 3.

The *in vitro* antibacterial activity of the prodrug was found to be similar to the parent drug as equal zones of inhibition were obtained against all the test organisms studied. This shows that there was no decrease in the antibacterial activity due to the condensation of formaldehyde to enoxacin. The minimum inhibitory concentration of the prodrug was also found to be the same as that of parent drug i.e. for *e. coli* 0.2 μg/ml[7]. However, since the rate of conversion of prodrug to the parent compound is very fast, it is also possible that the prodrug has converted to enoxacin thereby giving equal MIC or equal zones of inhibition. The above results indicated that the prodrug has enhanced solubility and dissolution rate and gets easily converted to the parent drug.

ACKNOWLEDGEMENTS

The authors thank Dr. M. Varshney (Dept. of Chemistry) for help in kinetic studies and to Dr. M. Ali (Dept. of Pharmacognosy and Phytochemistry) for spectral analysis. We are also grateful to Parke Davis & Co., Freiburg, Germany for the generous gift of drug sample.

REFERENCES

1. Henwood, J.M. and Monk, J.P. **Drugs.**, 1988, 36, 32.
2. Ross, D.L. and Riley, C.M., **Int. J. Pharmaceutics.**, 1990, 63, 237.
3. Bundgaard, H. and Johansen, M. **Arch. Pharm. Chem. Sci. ed.**, 1980, 8, 144.
4. Ramananthan S. and Agarwal, S.P. **Indian Drugs.**, 1993, 30, 571.
5. Swaminathan, A.G. and Agarwal, S.P. **Indian J. Pharm. Sci.**, 1993, 55, 159.
6. Goto, S., Jo, K., Kawakita, T., Kosaki, N., Mitsuhashi, S., T., Ohsawa, N. and Tanami, H. **Chemotherapy.**, 1981, 29, 76.
7. Matsumoto, J., Miyamoto, T., Minamida, A., Nishimura, Y., Egawa, H. and Nishimura, H. **J. Med. Chem.**, 1984, 27, 292.

Form (IV) See Rule (8)

Statement of ownership and other particulars as required under rule 8 of the Registration of Newspaper's (Central) Rules 1958.

1.	Place of Publication	:	Indian Pharmaceutical Association, Kalina, Santacruz (E), Mumbai - 400 098.
2.	Periodicity of its publication	:	Once in every two months
3.	Printer's Name	:	C.L. Kaul, for the Indian Pharmaceutical Association
	(Whether citizen of India)	:	Yes
4.	Address	:	C/o. Indian Pharmaceutical Association, Kalina, Santacruz (E), Mumbai - 400 098.
5.	Editor'sName	:	C.L. Kaul
	(Whether citizen of Indi)	:	Yes
6.	Address	:	C/o. Indian Pharmaceutical Association, Kalina, Santacruz (E), Mumbai - 400 098.
7.	Name and Address of individuals who own the Newspaper and partners or shareholders holding more than on per cent of the total capital	:	Indian Pharmaceutical Association, Kalina, Santacruz (E), Mumbai - 400 098.

I, C.L. Kaul hereby declare that the particulars given above are true to the best of my knowledge and belief.

Dated 30th March, 1997

Sd/-
(C.L. Kaul)