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Ofloxacin is a pyridone carboxylic acid derivative of nalidixic acid and differs from nalidixic acid by the addition of fluorine, an N-methylpiperazine, and an additional oxazine ring. This makes it a tricyclic quinolone. Its chemical (IUPAC) name is \((RS)-7\text{-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclo[7.3.1.0^{5,13}]trideca-5(13),6,8,11-tetraene-11-carboxylic acid.}\) It is possible to ascribe each of the different structural components of ofloxacin to a particular function (1).

Fluoroquinolones are bactericidal in activity, act on subunit A of DNA gyrase (bacterial topoisomerase), an enzyme that introduces negative super-twists into DNA and separates interlocked DNA molecules (2). This leads to interference with DNA replication, segregation of bacterial chromosomes, transcription, and other cellular processes. Bacterial resistance to the newer fluoroquinolones occurs less frequently than to the older analogue, nalidixic acid (3).

Ofloxacin has a broad spectrum of activity against Gram negative and Gram positive bacteria with poor activity against anaerobes (5). The ofloxacin minimum inhibitory concentration (MIC) for 90% (MIC90) of Enterobacteriaceae isolates (range 0.6 to 4 mg/L) would indicate inferior activity compared with ciprofloxacin (6). This may not be clinically significant since ofloxacin achieves higher serum concentrations. Gram positive bacteria are similarly sensitive to ofloxacin and ciprofloxacin, with Staphylococci species more sensitive than Streptococci species. As with other available fluoroquinolones, Streptococci are only moderately sensitive to ofloxacin with MIC values ranging from 1 to 4 mg/L (7).

Various types of binders are used in pharmaceutical industries extensively, especially in the

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**THE EFFECT OF BINDERS ON THE BIOAVAILABILITY OF OFLOXACIN TABLETS IN ANIMAL MODEL**

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**Abstract:** Present study was undertaken to evaluate the effect of binders on the bioavailability of the drug. Two formulations of ofloxacin were manufactured with two different binders, i.e. gelatin and starch, which were analyzed by different in vitro tests such as dissolution test using USP apparatus II (paddle method) by using 0.1 M HCl solution. For in vivo studies, blood samples were collected through the heparinized syringe at zero time (before dosing) and at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0 hours after the dosing of ofloxacin tablets to 24 rabbits and analyzed by high performance liquid chromatography. Mobile phase consisted of distilled water, acetonitrile and triethylamine (700 : 300 : 1.4, v/v/v). The pH of the mobile phase was adjusted at 2.4 with orthophosphoric acid. The maximum plasma concentration attained after the administration of formulation 1 (containing gelatin) was 7.56 ± 0.835 µg/mL (the mean ± SEM) and of formulation 2 (containing starch) was 3.4417 ± 1.16 µg/mL (the mean ± SEM). There is also statistically significant difference between the volume of distribution and total body clearance of both formulations. Therefore, formulation 1 is more bioavailable than formulation 2. Thus it can be concluded that binder can affect the bioavailability and pharmacokinetics of a drug.

**Keywords:** gelatin, starch, bioavailability, ofloxacin, pharmacokinetics

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development of tablet dosage form. It is therefore, necessary to find their influence on the bioavailability of a drug. Thus present study was designed as an attempt to investigate the effect of gelatin and starch on the bioavailability of ofloxacin in 24 rabbits.

MATERIALS AND METHODS

Chemicals and reagents

Ofloxacin was kindly gifted by Aventis Pharma, Pakistan. Carboxymethyl cellulose, methylene chloride and hydroxypropylmethyl cellulose were purchased from BDH, Germany. Gelatin, starch, magnesium stearate, talc, propylene glycol, ethyl alcohol, acetonitrile and triethylamine were purchased from Merck, Germany. Lactose (Riedel, Holland), disodium hydrogen phosphate (Sigma, Germany) and cellulose acetate phthalate (Fluka, Switzerland) were purchased through commercial sources.

Preparation of formulations

Two batches of ofloxacin 200 mg tablets (300 tablets each) were prepared using two different binders, i.e. gelatin (formulation 1) and starch (formulation 2), by wet granulation method with single punch machine (Neda, Pakistan). For the manufacturing of a batch of 300 tablets, ofloxacin (60 g) and lactose (55.65 g) were weighed and passed through sieve no. 16 individually and were placed in a tray; 8.1 g binder (gelatin or starch) was weighed and suspended in 10 mL of distilled water. About 30 mL of water was boiled separately. The suspension of the gelatin was added in the boiling distilled water until paste was formed. The gelatin paste was added in the dried ingredients and mixed for 15 min until wet mass was formed. This semi-solid mass was passed through the sieve no. 8 to produce granules. The granules were dried in dry oven at 50°C for 3 h. The dried granules were passed through sieve no. 10. Magnesium stearate (2.25 g), talc (1.5 g), corn starch (9 g) and carboxymethyl cellulose (1.5 g) were added in the granules for final mixing. The tablets were compressed by single punch machine. Both formulations were subjected to in-vitro and in-vivo tests under the same conditions for evaluation of significant difference in release profile as well as pharmacokinetic and bioavailability parameters.

Assay of tablets

Tablets of each formulation were triturated in a mortar to fine powder form. 100 mg of the powder was then dissolved in 100 mL of 0.1 M HCl. The solution in the flask was filtered and 1 mL of this solution was pipetted out in 100 mL volumetric flask. Volume was made up to 100 mL with 0.1 M HCl and the contents of ofloxacin were determined using spectrophotometer at a wavelength of 294 nm. The analysis was conducted in sets of six results and the average was then calculated.

Disintegration studies

In-vitro disintegration of both formulations was determined using USP disintegration apparatus (Neda, Pakistan) using water as disintegration medium.

Dissolution studies

In-vitro ofloxacin release was determined using USP dissolution apparatus-II (Curio, Pakistan) for both formulations using 0.1 M HCl as dissolution medium at temperature 37 ± 1°C. Paddle speed was set at 100 rpm.

In-vivo studies

Experimental work was performed at the Department of Pharmacy, the Islamia University of Bahawalpur, Pakistan. Bioavailability study of ofloxacin was conducted in 24 healthy rabbits (divided into two groups) following single dose cross over design with one week washout period.

Sample collection

Blood samples (each of 3 mL) were collected through the heparinized syringe at zero time (before dosing) and at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0 hours after dosing of the ofloxacin tablets. Blood samples were centrifuged at 3500 rpm for 10 min. The plasma was then harvested and frozen at -20°C until assay was performed. Samples were kept refrigerated during the period of collection.

Mobile phase preparation

Mobile phase consisted of distilled water, acetonitrile and triethylamine (700 : 300 : 1.4, v/v/v). Its pH was adjusted to 2.4 with orthophosphoric acid. Mobile phase was filtered under vacuum pressure of 150-200 torr using 0.45 µm membrane filters and degassed by flushing it with nitrogen for 2-3 min until complete degassing of the mobile phase was ensured.

Stock solution

Ofloxacin stock solution (1 mg/mL) was prepared freshly on daily basis by dissolving the drug in methanol.
Preparation of standard curve

Standard curve was constructed to encompass the anticipated range of plasma ofloxacin concentration found in healthy rabbits taking ofloxacin. Blank plasma was spiked with ofloxacin drug solution to give the concentrations of 0.5, 1, 2, 4, and 8 µg/mL. The extraction procedure was the same as described below. Injections of 20 µL were injected and spectra were taken of each concentration. The peak areas were noted for each concentration. The absolute recovery of ofloxacin from the extraction procedure was determined at different plasma concentrations (0.5 to 8 µg/mL) by comparing the peak heights of the drug obtained from extracted plasma samples with those obtained from direct injections of the pure ofloxacin standards in solvent of equivalent amounts.

Extraction procedure

Extraction procedure was simply based on precipitation method. 0.5 mL of drug solution was spiked with 0.5 mL of blank plasma in 2 mL centrifuge tube and centrifuged for 10 min. Organic layer was separated by micropipette, filtered using the filtration syringe and the filtrate were taken in the polypropylene tubes. 20 µL sample was injected into the HPLC apparatus injection port by injection syringe.

Instrumentation

Analysis was performed using HPLC apparatus (Perkin Elmer, 200 Series) and UV detector was set at 294 nm. A reverse phase system was used consisting of a 5 µm Hypersil ODS-C18 column (250 mm × 4.6 mm) preceded by 5 µm Hypersil ODS-C18 (10 mm × 4.6 mm) cartridge guard column.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using MS Excel Windows Professional XP and software “Kinetica 4.4”. Pharmacokinetic analysis was performed using non-compartmental method of analysis. Maximum concentration of ofloxacin in plasma (Cmax) and times of occurrence for maximum drug concentration (tmax) were determined by visual inspection of plasma concentration time profiles. At each time points (t), (C/t/Cmax) × 100 per individual was calculated and the maximum, median and minimum values for all subjects were determined. These percentages can provide some guidance regarding sampling times that can be used clinically. The area under the concentration time curve from 0 hour to infinity (AUC∞) was calculated by the linear trapezoidal rule using AUC from 0 hour to last measured concentration (Clast) plus Clast/Kel, where t is the time of last measured concentration and Kel is the terminal elimination rate constant. Statistical analysis was performed using SPSS (Self-Propelled Semi-Submersible) 12 version. Paired t-test was used to check the difference between the two formulations.

RESULTS AND DISCUSSION

In-vitro studies

Percentage of active ingredients of both formulations was calculated and analyzed by UV spectrophotometric method. The percentage of active
ingredients in both formulations was found to be 101.22% in formulation 1 and 102.15% in formulation 2. This is in accordance with B.P. (2004) specifications. Mean disintegration time for formulation 1 and formulation 2 was found to be 8 min and 11 min, respectively. The difference in the mean disintegration time of two formulations may be due to difference in the binders. Similarly, hardness of formulation 1 and formulation 2 was 7 kg/cm² and 5 kg/cm², respectively. Hardness of formulation 1 was found to be higher than formulation 2 as gelatin has higher binding properties than starch.

Dissolution profiles of both the formulations are shown in Figure 1. Rate of ofloxacin release from formulation 1 was found to be slightly faster than formulation 2, which might be due to the presence of gelatin. In spite of the fact that gelatin has higher binding power as compared to starch, it has liberated drug more quickly than starch.

**In-vivo studies**

The values for the mean plasma concentration of formulation 1 and 2 versus time were compared statistically by using paired *t*-test to observe the difference between two formulations and are given in Table 1 and shown graphically in Figure 2.

No statistically significant (*p* > 0.05) difference was observed between the AUC of both formulations. MRT (the mean ± SEM) of formulation 1 and formulation 2 was found to be 5.418 ± 0.815 h and 5.548 ± 0.815 h, respectively. There is no statistically significant (*p* > 0.05) difference in the MRT values of both formulations. In a previous study conducted by Kenneth et al. on healthy human volunteers, half-life was found in the range of 5-8 h after the dose of 200 mg of ofloxacin (8). In the present study, the plasma half life (the mean ± SEM) of formulation 1 was 3.755 ± 1.24 h and formulation 2 was 3.845 ± 0.979 h. No statistically significant (*p* > 0.05) difference was found between the half-lives of both formulations. The elimination of a drug is usually an exponential (logarithmic) process so that a constant proportion of the drug in the body is eliminated per unit of time. Therefore, the elimination rate constant (*K*ₐ) is the proportionality constant expressing the proportion of drug in the body eliminated per unit of time. In this study, *K*ₐ of formulation 1 ranged from 0.153 ± 0.191 to 0.273 ± 5.037 and for the formulation 2 ranged from 0.1247 ± 0.192 to 0.2711 ± 4.44. There is no statistically significant (*p* > 0.05) difference between the *K*ₐ of both formulations. The volume of distribution (*V*ₜ) is the volume in which drug is assumed to be uniformly distributed (9). In a previous study conducted by Kenneth et al. on human volunteers, apparent *V*ₜ of the drug was reported to be 1.0-1.5 L/kg with an oral dose of 200 mg of ofloxacin (8). In our study, the mean ± SEM value of *V*ₜ for formulation 1 was 31.25 ± 0.429 L/kg and for formulation 2 was 59.33 ± 0.227 L/kg. These values are greater than the values reported in the previous studies conducted on healthy human volunteers. The difference in the values of volume of distribution may be due to the difference in species.

The plasma concentration of ofloxacin (formulation 1 and 2) was utilized to calculate the pharmacokinetic parameters in individual animals. The pharmacokinetic parameters were calculated using non-compartment model of analysis and were compared statistically. In the present study, the mean (± SEM) maximum plasma concentrations (*C*ₘₚ) for formulation 1 and formulation 2 were 7.56 ± 0.835 µg/mL and 3.4417 ± 1.16 µg/mL, respectively. These values are found higher than the already
reported values on human beings, where maximum plasma concentration (C_{max}) was found to be 1.6-2.2 mg/L with an oral dose of 200 mg, 3.2-4.3 mg/L after an oral dose of 400 mg and 6.7-8.1 mg/L with an oral dose of 600 mg of ofloxacin (8). These differences might be due to difference of species and the difference in the formulations. C_{max} for formulation 1 is very significantly (p < 0.01) higher than that of formulation 2 what means that the formulation 1 has better bioavailability than formulation 2. The time of peak plasma concentration (t_{max}) corresponds to the time required to reach maximum drug concentration after drug administration. At t_{max}, peak drug absorption occurs and the rate of drug absorption exactly equals to the rate of drug elimination (9).

In a previous study conducted by Wise and Lockley on human volunteers, ofloxacin had t_{max} value of 0.5 to 3 h (10). In another study conducted by Tunkel et al. on healthy young volunteers, t_{max} values were reported to be 1.6 ± 1.2 h after an oral dose of 100 mg, 1.2 ± 0.4 h after an oral dose of 300 mg and 1.2 ± 0.6 h after an oral dose of 600 mg of ofloxacin (11). In the present study, t_{max} of formulation 1 and formulation 2 was found to be 2.0 h in all rabbits. This value is slightly greater than those previously reported. The higher value might be due to lower body weight and blood volume of rabbits as compared with human beings, since in human beings increasing the dose increases the value of t_{max} (12).

In a previous study, the area under the curve (AUC_{0-\infty}) was found to be 14.6 µg◊h/mL and 28 µg◊h/mL with an oral dose of 200 mg and 400 mg of ofloxacin (8). Tunkel et al. reported a value of AUC as 9.0 ± 0.9 µg◊h/mL, 53.8 ± 7.9 µg◊h/mL and 27.5 ± 6 µg◊h/mL with an oral dose of 100 mg, 300 mg and 600 mg of ofloxacin, respectively, in healthy human young volunteers (11). In the present study the mean ± SEM value of AUC_{0-\infty} of formulation 1 was 23.2157 ± 0.3864 µg◊h/mL and of formulation 2 was 22.1868 ± 1.1607 µg◊h/mL. No statistically significant (p > 0.05) difference was observed between the values of AUC_{0-\infty} of both formulations. The clearance is one of the parameters that determines the rate of maintenance dose required to achieve a target plasma concentration and therefore, effect at the steady state. In the present study, the mean ± SEM value for the total body clearance (Cl) of formulation 1 was 5.865 ± 0.938 mL/h/kg and 10.92 ± 0.5142 mL/h/kg for formulation 2. There is statistically highly significant (p < 0.01) difference between the Cl of both the formulations. In the previous studies (8) the clearance values were found to be 230 mL/min after an oral dose of 200 mg and 240 mL/min after an oral dose of 400 mg of ofloxacin.

**CONCLUSION**

The results of the study show that the formulation variables such as a change in the binders, e.g. gelatin and starch, affect the availability of the drug. Similarly, the effect of other excipients on the formulation and subsequent bioavailability of the drug should be determined

**REFERENCES**


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