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The determination of ofloxacin in human skin tissue by high-performance liquid chromatography and correlation between skin tissue concentration and serum level of ofloxacin

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Abstract—A simple high-performance liquid chromatographic (HPLC) assay was developed for the measurement of ofloxacin in skin tissue, and the correlation between serum levels and skin tissue levels of ofloxacin was determined. The homogenized skin samples were directly introduced into an HPLC system after filtering through a Molcut II membrane filter to remove proteins. The filtrate was concentrated on a pre-column using a phenyl-stationary phase and was then introduced to an analytical column using an ODS-stationary phase by column-switching techniques. Ofloxacin and lomefloxacin as an internal standard were detected by UV absorbance at 300 nm. Determination was possible for ofloxacin over the concentration range 250–3000 ng (g skin tissue)$^{-1}$; the limit of detection was 100 ng g^{-1}. The absolute recovery of ofloxacin added to skin tissue homogenate was over 72% with a coefficient of variation of less than 4·1%. This method is applied to determination of skin tissue level of ofloxacin in patients after treatment with ofloxacin. The correlation between serum levels and skin tissue levels of ofloxacin was determined for 30 patients after oral administration of ofloxacin. Good correlation was obtained and the coefficient of correlation was 0·84.

Ofloxacin, 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1.2,3-de]-1,4-benzoxazine-6-carboxylic acid (Fig. 1), is a fluoroquinolone that shows a good activity against Gram-negative and Gram-positive bacteria in-vivo and in-vitro (Sato et al 1982). The mechanism of effect is based on the inhibition of the DNA-gyrase of the bacteria. The extensive distribution of ofloxacin in the tissues, including skin of the rat, has been reported after oral administration (Okazaki et al 1984). Therefore, it is considered that the oral administration of ofloxacin would be effective for secondary wound infection. The correlation between serum levels and skin tissue levels of ofloxacin was reported in rat (Umemura & Nohara 1984; Yamamoto et al 1984) and man (Tomizawa et al 1984; Takahashi et al 1984). These previous papers have reported only seven (Tomizawa et al 1984) and three (Takahashi et al 1984) cases of skin tissue level of ofloxacin in patients and the coefficients of correlation were not given.

A microbiological assay of ofloxacin has been described (Lockley et al 1984). Assay methods such as this have the disadvantage of a poor limit of detection and a lack of selectivity.

Several workers have reported high-performance liquid chromatography (HPLC) methods for the determination of ofloxacin in body fluids (Mignot et al 1988; Matsubayashi et al 1989; Okazaki et al 1991). In our previous report, we described a method for direct injection of serum using a column switching technique (Ohkubo et al 1992). This method is useful for the determination of ofloxacin in serum in clinical drug monitoring.

In the present paper, we describe a direct injection HPLC method using the column-switching technique without extraction for the determination of skin tissue level and serum level of ofloxacin in patients after oral treatment with ofloxacin.

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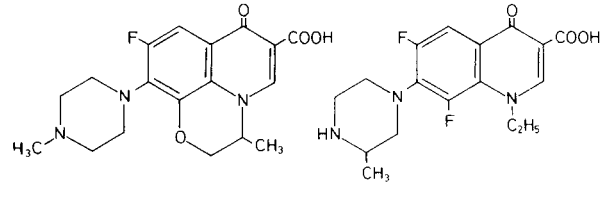
Materials and methods

Chemicals and materials. Ofloxacin and lomefloxacin hydrochloride (Fig. 1) were kindly donated by Daiichi Seiyaku Co. Ltd (Tokyo, Japan) and Shionogi Pharmaceutical Co. (Osaka, Japan), respectively. Molcut II membrane filter was obtained from Millipore Co. (Bedford, MA). All solvents used were of HPLC grade (Wako Pure Chemicals Industries Ltd, Osaka, Japan). All other reagents and chemicals were purchased from Wako Pure Chemicals Industries Ltd, or Nakarai Tesque Inc. (Kyoto, Japan). All reagents used were analytical grade.

Instruments. The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco Co. Ltd, Tokyo, Japan) equipped with a Uvidec 100-VI ultraviolet detector (Jasco) set at 300 nm. Test samples were injected using a Model 7125 injector (Rheodyne Inc., Cotati, CA) with an effective volume of 500 μL, a Develosil Ph-5 (Nomura Chemical Co., Seto, Japan) pre-column and a Develosil ODS-5 analytical column. The stainless-steel analytical column (150 × 4·6 mm i.d.) was packed in our laboratories by a conventional high-pressure slurry-packing procedure. The pre-column (50 × 4·6 mm i.d.) was packed by low pressure packing technique. The mobile phase consisted of 0·5% sodium acetate-acetonitrile (87:13, v/v), pH adjusted with 50% phosphoric acid before mixing, and degassed ultrasonically.

Procedure for the determination of ofloxacin in skin. Skin tissue. Skin tissue was cut into 2 or 3 mm^2 pieces and homogenized in a Polytron homogenizer (Kinematica AG, Littau, Switzerland) with a solution of 0·5% sodium acetate (pH 2·5)-acetonitrile (87:13, v/v) (2 mL) containing lomefloxacin as an internal standard (2400 ng). The homogenized medium was deproteinized by filtration through a Molcut II membrane. The filtrate (50 μL) containing ofloxacin and internal standard was then loaded on the pre-column for elimination of extraneous substances in the human skin tissue homogenate. After washing for 3·0 min on the pre-column, ofloxacin and lomefloxacin were eluted from the pre-column and then led to the analytical column by a column switching technique using 0·5% sodium



Ofloxacin Lomefloxacin

FIG. 1. Chemical structures of ofloxacin and lomefloxacin.

acetate (pH 2·5)-acetonitrile (87:13, v/v) as the isocratic mobile phase.

Serum. Lomefloxacin (1000 ng) in methanol (10 μL) was added to serum samples (500 μL) as an internal standard and then 100 μL acetonitrile was added. The subsequent procedure was as previously described (Ohkubo et al 1992).

Calibration curves. Known amounts of ofloxacin in the ranges of 250–3000 ng g^{-1} and 50–2000 ng mL^{-1} were added to the blank skin tissue homogenate and the blank serum, respectively. These skin tissue and serum samples were treated according to the determination procedure described above. The peak-height ratio of ofloxacin and lomefloxacin were measured and plotted against the known concentration of ofloxacin.

Drug administration and sampling. Ofloxacin (200–300 mg as a tablet) was orally administered to patients for prevention of secondary wound infection during surgery. Venous blood samples (2·5 mL) were collected from patients (who had given informed consent) at 0·5–3 h after the administration. Sera were separated by centrifugation at 1900 *g* for 15 min and stored at −45°C until analysis. Skin samples, including scar tissue, haemangioma, granulation tissue, burned skin and healthy skin around the tumour were obtained from a part of the excised skin.

Results and discussion

Initially, our efforts were directed towards developing an efficient chromatographic system for the direct analysis of ofloxacin in skin using column switching techniques. In our previous paper, we studied the effects of the pH of the mobile phase on the capacity factor values of ofloxacin, norfloxacin, ciprofloxacin and enoxacin using six kinds of stationary phase (Ohkubo et al 1992). From these results, we chose enoxacin as a suitable internal standard. The separation of endogenous substance and new quinolone derivatives was satisfactory when a phenyl pre-column and an octadecyl analytical column with a 0·5% sodium acetate (pH 2·5)-acetonitrile (87:13, v/v) mobile phase system were employed (Ohkubo et al 1992). In the present paper, we studied the characterization of lomefloxacin, tosfloxacin and sparofloxacin on the six kinds of stationary phase. In all these compounds, the k values increased with increasing pH in the range 3·5–4·5 with 0·5% sodium acetate-acetonitrile (87:13, v/v). These quinolone derivatives were retained more strongly on phenyl, *tert*-butyl and octadecyl stationary phases. Lomefloxacin could be easily separated using these mobile phases over the entire pH range. The best resolution, peak sharpness and suitable analytical time was obtained with lomefloxacin with these stationary phases, and lomefloxacin was therefore selected as the most suitable internal standard for the determination of ofloxacin in skin tissue homogenate. A combination of a pre-column and an analytical column was selected so that interfering endogenous substances and the quinolone derivatives had different interactions with the two columns. Finally, we established a satisfactory chromatography system using lomefloxacin as an internal standard, phenyl stationary phase as a pre-column and octadecyl stationary phase as an analytical column and a 0·5% sodium acetate (pH 2·5)-acetonitrile (87:13, v/v) mobile phase system. There are several reports describing the techniques for the determination of ofloxacin in body fluids involving extraction and purification steps (Mignot et al 1988; Matsubayashi et al 1989; Okazaki et al 1991). In these reports, however, satisfactory results were not obtained due to the tedious extraction procedure and poor extraction yield. Therefore, our direct injection method with a column switching technique was satisfactory with respect to simplicity and accuracy. Extraction of ofloxacin from water and other buffer solutions was poor; in the present study, we used 0·5% sodium acetate (pH 2·5):acetonitrile (87:13, v/v) as an extraction medium, from which we obtained a high recovery of ofloxacin from skin tissue homogenates. Fig. 2 shows representative chromatograms for a blank human skin tissue homogenate sample, a human skin tissue homogenate sample obtained from a patient treated with ofloxacin, a blank serum sample and a serum sample obtained

Table 1. Analytical recovery and between-assay precision of ofloxacin in skin tissue homogenate.

Added (ng g^{-1})	Absolute recovery (%) (mean ± s.d.)	Coefficient of variation (%)
500	84·5 ± 3·9	4·6
1000	74·5 ± 2·9	3·9
3000	74·3 ± 1·6	2·2

n = 4.

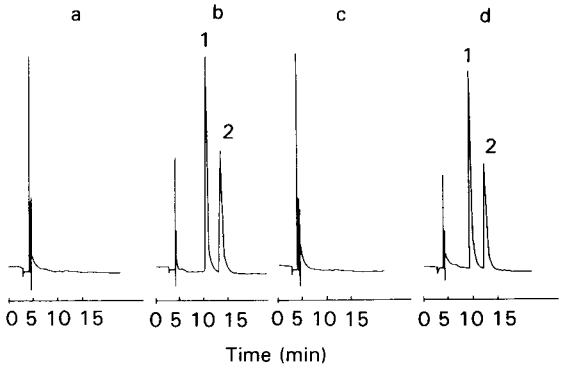


FIG. 2. Chromatograms of a: blank skin tissue homogenate, b: skin tissue homogenate in patients after treatment with 200 mg ofloxacin, c: blank serum, and d: serum in patients after treatment with 200 mg ofloxacin. 1 = ofloxacin, 2 = lomefloxacin.

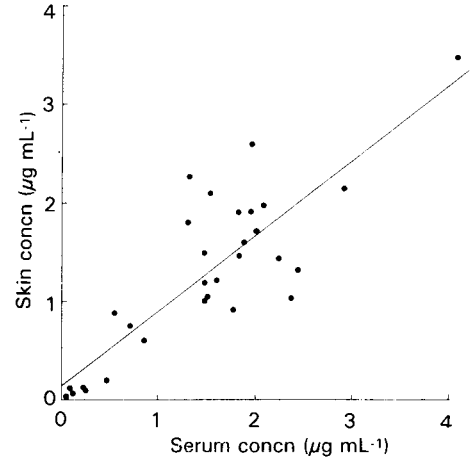


FIG. 3. Relationship between concentration of ofloxacin in skin tissue and ofloxacin in serum after oral administration of ofloxacin.

from a patient treated with ofloxacin. No significant interfering peaks derived from biological substances were seen on the chromatograms.

Calibration graphs for ofloxacin in human skin tissue and serum were linear over the ranges 250–3000 ng g^{-1} and 50–2000 ng mL^{-1}, respectively. The limits of detection for ofloxacin were 100 ng g^{-1} in skin tissue and 20 ng mL^{-1} in serum. The results of recovery studies are given in Table 1. The coefficient of variation for the concentrations of ofloxacin in the patients samples ranged from 0·4 to 1·6%. The concentration of ofloxacin in skin tissue samples and serum samples was determined in patients receiving 200–300 mg ofloxacin by mouth before surgery. Fig. 3 shows that the correlation between results with skin tissue ofloxacin (y) and serum ofloxacin (x) was very good, giving the regression: $y = 0{\cdot}785x + 126{\cdot}9$ ng mL^{-1} ($r = 0{\cdot}84$, $n = 30$). The average concentration ratio of ofloxacin in skin tissue vs serum was 0·84. Several workers have reported serum and skin tissue levels of ofloxacin in man (Tomizawa et al 1984; Takahashi et al 1984). However, no coefficient of correlation was obtained in this paper, because the sample number was not sufficient for statistical treatment. Our results suggest that the therapeutic basis of treatment of secondary wound-infection by oral administration of ofloxacin is firmly established in man. In conclusion, a simple, practical and accurate HPLC method has been developed for the determination of ofloxacin in human skin tissue and serum without prior extraction. The good distribution of ofloxacin from blood to skin tissue after oral administration using our method is useful for pharmacokinetic studies in skin tissue and serum in patients.

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Moment analysis of hepatic local disposition of allopurinol and oxipurinol: metabolism kinetics from allopurinol to oxipurinol in the rat isolated perfused liver

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Abstract—Drug metabolism in the liver was examined by the rat isolated perfused liver using the single-pass bolus-input technique. The test compounds, allopurinol and its metabolite oxipurinol, were independently introduced into the liver from the portal vein, and the concentration profiles in the venous outflow were monitored and kinetically analysed by moment theory. The recovery ratios of allopurinol and oxipurinol after the individual administration of each drug were estimated to be 0·17 (±0·08 s.d.) and 1·03 (±0·02 s.d.), respectively. The outflow recovery ratio of oxipurinol as the metabolite after allopurinol administration was estimated to be 0·80 (±0·07 s.d.). These results indicate that the combined outflow recovery of the precursor and the metabolite after allopurinol administration is almost 100% in the rat liver.

Allopurinol and oxipurinol have been used extensively as potent inhibitors of xanthine oxidase, an enzyme that converts hypoxanthine to xanthine, and xanthine to uric acid, for the medical treatment of hyperuricaemia and gout (Elion et al 1963; Rundles et al 1963; Elion 1966; Hille & Massey 1981). Allopurinol is metabolized by xanthine oxidase in the liver to oxipurinol (Elion et al 1966; Krenitsky et al 1967). There are very few reports on the hepatic local disposition of allopurinol and oxipurinol, the target organ of which is the liver. The purpose of this study is to evaluate in detail the local disposition of allopurinol and oxipurinol in the liver, and to elucidate the metabolic kinetics from allopurinol to oxipurinol by means of single-pass rat-liver perfusion experiments following impulse administration into the portal vein. Several models for the analysis of both local disposition and metabolism kinetics during an organ pass have been proposed of which the well-stirred model and the parallel-tube model (Pang & Rowland 1977) are the simplest. A dispersion model has also been used in the analysis of the outflow dilution curve following an impulse input (Roberts &

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