Disposition of quinolones

= Comparison of a novel quinolone PZFX with other new quinolones =

Introduction

Since the discovery of nalidixic acid(NA), quinolones have made great progress in antimicrobial activity and pharmacokinetic properties. First-generation quinolones such as NA, oxolinic acid(OA) and piromidic acid(PA) were well absorbed after oral administration, but due to lipophilicity, these old quinolones were easily metabolized and poorly distributed to tissues. The new quinolones developed since the discovery of NFLX can be absorbed after oral administration and have additional advantageous pharmacologic properties including excellent distribution to many tissues and metabolical stability. However, these new quinolones tend to show low serum concentrations.

A novel quinolone PZFX demonstrated a high Cmax and short T1/2 in experimental animals and humans compared with those of other new quinolones such as SPFX or OFLX . An in vitro antimicrobial study of PZFX showed that, when the product of concentration by time remained constant, high-concentration short-term exposure was superior to low-concentration long-term exposure. Therefore, PZFX is likely to induce higher antimicrobial activity in vivo. Since the efficacy of PZFX in vivo was considered due to its high Cmax, we investigated why the Cmax of PZFX is high. Based on our findings, I would like to present our company's thinking about the advantageous disposition of quinolones.

Experiment

We chose SPFX and OFLX as reference drugs because OFLX has a rather high serum concentration among commercially used quinolones and SPFX is another type of quinolone with a low serum concentration and long T1/2.

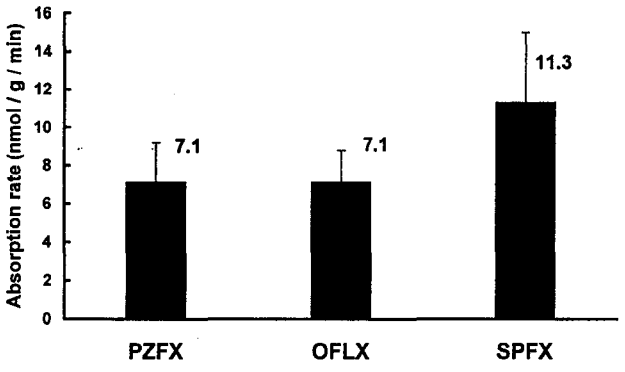
1) Absorption rate and mechanism : 0.5 μ mol of drug with or without inhibitor (Glycylglycine 100 μ mol, Nicotinic acid 10 μ mol) was injected into a rat jejunal loop and the amount remaining in the loop after 10min was measured . 2) Estimation of renal and other clearance : Drugs were intravenously administered to rats (10mg/kg or 20mg/kg) that were intestinally perfused with isotonic phosphate buffer(pH=6.5). Drug concentrations in urine, bile, artery plasma and intestinal perfusate were measured and the clearance for each was calculated. 3) Renal excretion process by stop-flow analysis : Rabbits were infused with a solution consisting of the test drug, p-aminohippuric acid, creatinine and D-mannitol. Urinary flow was stopped by clamping the ureters, inuline was injected before removing clamp from the duct. Urine was fractionated to 0.25ml and drug concentration as well as the concentration of other markers were measured. 4) Tissue to serum concentration ratio(Kp) and contribution of tissue to Vdss. : Apparent Kp values were calculated from the drug concentration in tissue and in plasma after intravenous administration of PZFX to rats. Apparent Kp values of OFLX and SPFX were calculated from reference data. Contribution of tissue to Vdss was estimated from the Kp value calculated from tissue plasma flow, plasma concentration-time data, renal clearance and the elimination rate constant at the steady state. 5)Uptake clearance into muscle: Rats or rabbits were intravenously administered test drugs and plasma samples were collected over a very short period. At the

final sampling point, animals were sacrificed to obtain a muscle specimen. Uptake clearance was calculated from AUCs of plasma and drug concentration in muscle. 6)Tissue free fraction: Binding percent of drugs to rat muscle homogenate was measured with changing drug concentrations, tissue unbound fraction was estimated in undiluted homogenate. 7)The relation of unbound drug concentration between tissue interstitial fluid and plasma: A microdialysis probe was implanted in rat muscle to measure the unbound drug concentration in plasma and microdialysis perfusate after intravenous administration of PZFX. 8)Drug concentration at the infection site : Paper disks or a tissue cage was subcutaneously implanted in the back skin of rats. Drug concentration in exudate and serum were measured after oral administration .

Results

Absorption

We used rat jejunal loop to study the inhibitory effect of some reagents and to estimate the absorption rate, because all quinolones investigated here were well absorbed from the duodenum and jejunum. Glycil-glycine dramatically inhibited SPFX absorption and OFLX absorption was inhibited by nicotinic acid. While PZFX absorption was not affected by either reagent. The absorption mechanism might be distinguished in those three drugs, but the absorption rate of PZFX was almost the same as that of OFLX.(Fig.1) SPFX, which has the lowest Cmax, showed the highest absorption rate. The high Cmax of PZFX could not be explained by the absorption process.



0.2ml of drug solution (0.5 μ mol/ml) was injected into jejunum loop.
Jujunum loop was excised 10-15 min after injection and inner loop was washed with 0.01N HCl followed by water. PZFX n=5, OFLX n=3, SPFX n=6

Fig.1 Absorption rate of quinolones in rat jejunal loop.

Excretion

The excretion pattern of SPFX differed from those of PZFX and OFLX after intravenous administration to rats. All quinolones tested were slightly excreted into bile and were excreted from the intestine to intestinal lumen . PZFX and OFLX were mainly excreted to urine whereas SPFX was

mainly excreted by a non-renal pathway. The excretion of PZFX and OFLX from renal tissue showed net secretion, while renal excretion of SPFX showed net absorption.(Fig.2) Stop-flow analysis in rabbits suggested that PZFX and OFLX were both secreted to the proximal tubule, but PZFX was hardly reabsorbed from the distal tubule in contrast with OFLX which was well reabsorbed.(Fig.3) It is considered that this non-reabsorption system from the distal tubule of PZFX caused the rapid disappearance of PZFX from serum. SPFX was excreted by glomerular filtration only and reabsorbed from the distal tubule. Consequently, PZFX showed the highest value for renal clearance, while SPFX showed the lowest. PZFX which has high Cmax showed the highest renal clearance and total clearance of these three quinolones were almost the same, we could not explain the high Cmax of PZFX by the excretion study.

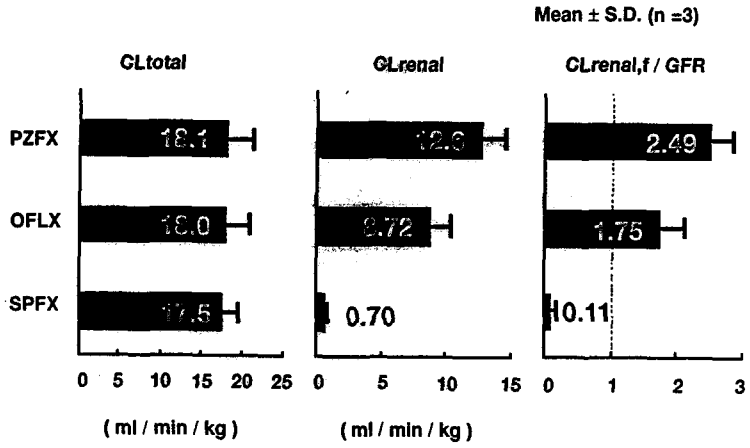


Fig.2 Clearance of PZFX,OFLX, and SPFX after intravenous administration (10mg/kg) to male rats.

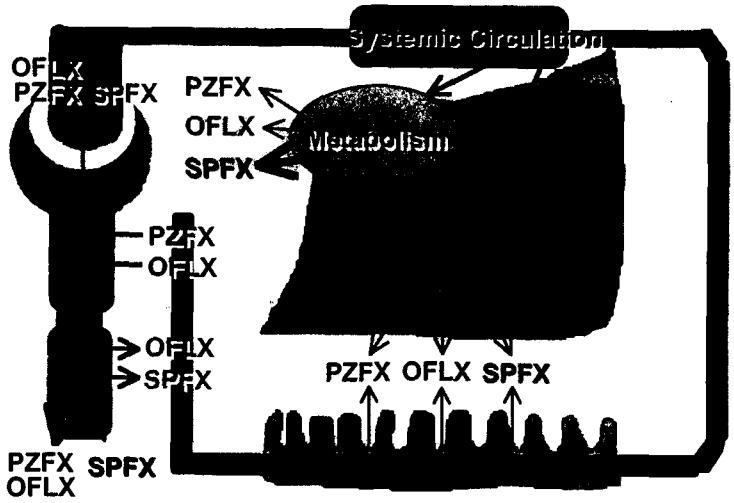


Fig.3 Elimination of new quinolones from body

Distribution

Vdss is a crucial parameter for the quinolone concentration-time profiles in rats serum. The concentration-time profiles in rats after oral administration were comparable with that simulated by a one compartment model with first order absorption using parameters of Vdss and total clearance after intravenous administratioin, and absorption rate constant estimated by the absorption rate from intestinal loop experiment. PZFX has a low Vdss which caused a high Cmax in serum, but the value was rather high compared with that of inuline, indicating that PZFX can penetrate into cells.

To estimate the contribution of each tissue to Vdss in rats, we calculated Kp values using renal and hepatic intrinsic clearance, tissue plasma flow, elimination rate constant at a pseudo-steady state after intravenous injection and the apparent Kp values of each tissue obtained from an ordinary tissue distribution study. PZFX had a relatively low apparent Kp value in many tissues compared with those of OFLX and SPFX. Muscle was the most important tissue determining the Vdss for these three quinolones. (Fig4)

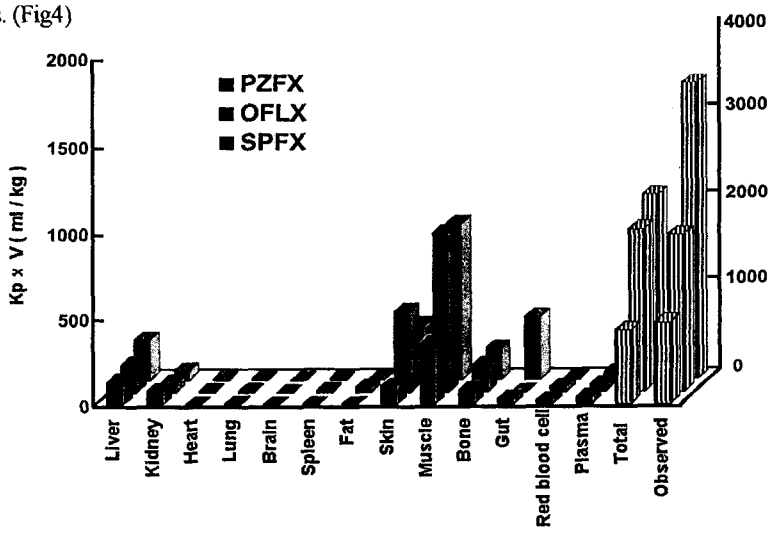


Fig.4 Contribution of each tissue to the volume of distribution at steady state.

Since the difference in Vdss among PZFX, OFLX and SPFX could not be explained by the unbound plasma fraction, we investigated the unbound tissue fraction using rat muscle homogenate. The unbound tissue fraction in muscle was comparable among the three drugs, therefore the difference in Vdss could not be explained by binding to the tissue component. We investigated the muscular uptake clearance of quinolones in rats and rabbits. PZFX showed a low uptake clearance similar to that of β-lactams.(Fig.5) It is considered that the transfer rate from capillary vessels to the inter cellular space was very rapid and there was no difference among the drugs. Therefore, the difference between PZFX and other quinolones on muscular uptake clearance is thought to be due to the difference in penetration clearance into the cells. It is still unclear whether the difference in muscular uptake clearance between PZFX and other quinolones might be related to the difference in Vdss. It can hardly be considered that there is some transport system in muscle. However, the large difference in muscular uptake clearance between PZFX and other quinolones is very interesting.

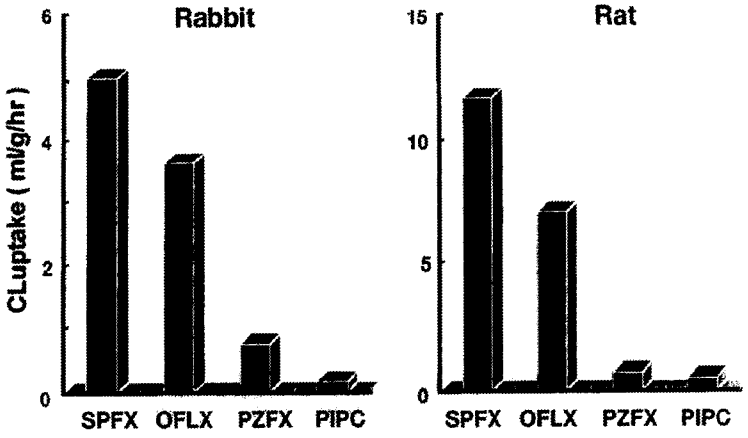


Fig.5 Uptake clearance of PZFX and other drugs into muscle.

Tissue distribution and efficacy

PZFX has low apparent Kp values and Vdss. Concentration of free drug at the infection site is of great importance, since most infections occur in the interstitial fluid of tissues and only the free concentration exerts a pharmacological effect. It is the free concentration in plasma that determines the unbound concentration of interstitial fluid and intra cellular fluid.

We measured the free concentration in interstitial fluid in muscle by the microdialysis method in rats. After intravenous injection, the free PZFX concentration in muscular interstitial fluid was equal to the free drug concentration in plasma.(Fig.6) We also investigated the concentration at the infection site using an experimental infection model in rats. PZFX showed a high concentration in the exudate from the animal model compared with those of OFLX and CPFX, reflecting the concentration in serum.

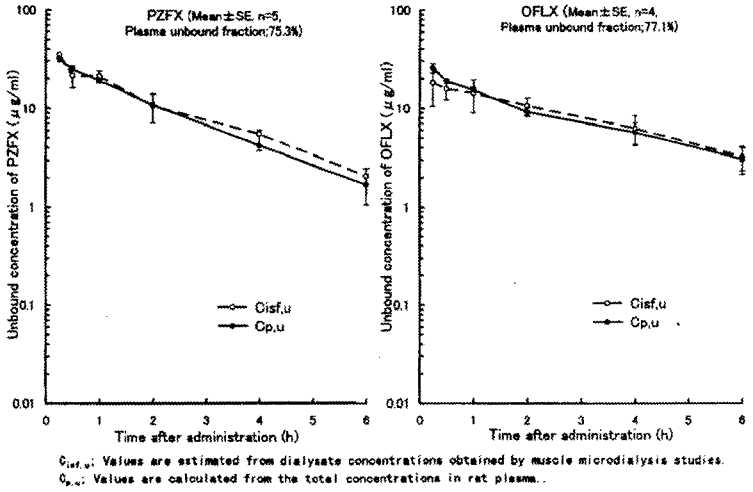


Fig.6 Unbound concentrations of PZFX and OFLX in muscle interstitial fluids(Cisf,u) and plasma (Cp,u) of rats. (50mg/kg ,i.v.)

Conclusion

The novel quinolone PZFX has a high Cmax in humans and experimental animals. It is considered that the excellent in vivo efficacy of PZFX is due to the high Cmax in serum. We investigated the PZFX disposition in comparison with those of OFLX and SPFX to clarify the high Cmax of PZFX.

The absorption mechanism may be distinguished in those three drugs, but the absorption rate of PZFX from rat jejunal loop was almost the same as that of OFLX. SPFX, which has the lowest Cmax, showed the highest absorption rate. PZFX and OFLX were mainly excreted to urine by secretion from the proximal tubule. Whereas SPFX was mainly eliminated by a non renal pathway, that is, metabolism. All three quinolones were excreted into the intestinal lumen from the intestine and excreted into bile, but the ratios of clearance by that route to total clearance were not large. PZFX showed the highest renal clearance, while total clearance of those three drugs were almost the same. We could not explain the high Cmax of PZFX by the absorption and excretion processes.

The high Cmax of PZFX was thought to be due to low Vdss. Vdss of the three quinolones were larger than that of inuline, suggesting that the quinolones penetrated into tissue cells. We investigated the detail of distribution of the three quinolones using rat muscle, because muscle is the most important tissue determining the Vdss. Unbound tissue fraction of those quinolones in muscle were comparable, therefore the difference in Vdss could not be explained by binding to tissue component. It is considered that the unbound drug concentration ratio of intra-cellar fluid to interstitial fluid might differ among PZFX,OFLX and SPFX. Uptake clearance into tissues of PZFX was much lower than those of other quinolones. Whether the difference of tissue uptake clearance was related to difference of the Vdss remains obscure, because we can hardly consider that some transport system exists in muscle. The slow uptake of PZFX into cells may be an advantage with regard to safety.

Based on these findings, PZFX concentration in the cell was thought to be low compared with that of other quinolones. However, unbound PZFX concentration in tissue interstitial fluid was higher than that of other quinolones. Because, the free drug concentration in tissue interstitial fluid was equal to that in plasma. Consequently, PZFX showed a high concentration at the infection site reflecting the concentration in plasma. It is thought to be more advantageous for quinolones to have a high unbound concentration in serum than to have an excellent tissue distribution, since most infections occur in the interstitial fluid of tissues.