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Effect of Induced Mastitis on Disposition Kinetics of Gatifloxacin Following Intravenous Administration in Goats

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Abstract

Disposition kinetic studies of gatifloxacin (GAT) was conducted after single i.v. dose (10 mg/kg) in six healthy and six mastitic Black Bengal lactating goats. Mastitis was induced by coagulase positive *S. aureus*. The concentration of the drug was estimated by HPLC. The maximum milk concentration was found to be significantly ($p< 0.05$) higher in mastitic goats (12.78 ± 3.11 µg/ml) than healthy (9.17 ± 1.41 µg/ml). The therapeutic milk concentration in mastitic goats (0.13 ± 0.05 to 12.41 ± 2.99 µg/ml) was maintained for 48 h which was significantly ($p<0.01$) longer than in healthy goats (24 h). The elimination half-life in plasma and milk of mastitic goats (5.82 ± 0.67 and 8.20±0.21 h) was significantly ($p<0.01$) higher than healthy (4.54 ± 0.75 and 3.67±0.09 h).It indicates that GAT persisted in the body of mastitic goats for a longer duration. The AUC_{milk}/AUC_{plasma} ratio was 5.82. The $t_{½}$ milk /$t_{½}$ plasma ratio was 1.41. MIC in this experiment was considered to be 0.1 µg/ml. The AUC/MIC ratio of plasma and milk of mastitic goats were 180 and 1049 respectively. On the basis of the results obtained it was concluded that GAT exhibited improved pharmacokinetic parameters with good penetration and longer persistence in mastitic milk, which will be of great help in the treatment of mastitis in goats caused by *S. aureus*.

Keywords: Disposition kinetics; Gatifloxacin; Goats; Mastitis; i.v.

Introduction

Gatifloxacin (GAT), a fourth generation fluoroquinolone, selectively inhibits bacterial enzymes DNA gyrase and Topoisomerase IV (Perry et al., 1999). Fluoroquinolones have some favourable characteristics such as large volume of distribution, low plasma protein binding, and relatively low MIC against susceptible target microorganisms (Brown, 1996). The MIC value of GAT against *Staphylococci* has been reported to be 0.1µg/ml (Tsurumaki et al., 2000; Boubakar et al., 2006). GAT has a chiral center in its structure, exists in plasma as two equi-active equi-proportional R- and S-enantiomers (Wise et al., 1999). Fluoroquinolones act by a concentration-dependent killing mechanism (Drusano et al., 1993), which is associated with a relatively prolonged postantibiotic effect (Aliabadi and Lees, 2001).

Mastitis is a worldwide problem among lactating animals from both economical and public health point of view. In most of the clinical cases of mastitis in goats *Staphylococcus aureus* is the causative agent (Shearer and Harris, 2008). *S. aureus* is susceptible to a variety of antibiotics in-vitro. However, the potential contributors to the poor response of *S. aureus* to antimicrobial in-vivo cure may be its ability to survive inside neutrophils (Yancey et al., 1991; Mullarky et al., 2001), and to invade into mammary epithelial cells (Kerro Dogo et al., 2002). The ability to survive phagocytosis by neutrophils protects the bacteria even if they are exposed to the host immune response, except in the case of antibiotics that penetrate intracellularly (Barkema et al., 2006). Fluoroquinolones are widely studied to display intracellular bioactivity against bacteria which reside and/or multiply within phagocytes (*Staphylococcus aureus, Legionella pneumophila*, mycobacteria, chlamydiae etc). For most of the fluoroquinolones the uptake by phagocytes is moderate and rapid (Loo et al., 1997; Memin et al., 1997). Thus it is expected that GAT would be effective in treating *S. aureus* mastitis. It has also been evidenced that mastitis has an effect on the milk concentrations of antimicrobials in goats (Sar et al., 2006). Limited pharmacokinetic parameters of GAT are available in healthy goats (Verma and Roy, 2006). However, pharmacokinetic parameters of GAT and its milk penetration especially in mastitic goats are not available.

Hence the presents study was undertaken to determine the pharmacokinetic study and milk penetration of GAT following single intravenous (i.v.) administration in healthy and mastitic goats.

Materials and Methods

Animals

Twelve clinically healthy Black Bengal lactating goats (14-20 kg) of 2 to 2.5 years were used in this study were provided green fodder, routine grazing (daily for six hours) and balanced ration (2 parts wheat husk, 1 part groundnut cake and 1 part crushed maize). Water was provided *ad libitum*. The mean temperature and relative humidity of experimental animal room were 22 – 28°C and 65 - 92%, respectively.

Drugs used

Gatiquin® infusion (Cipla pharmaceutical Ltd., India) was injected i.v. at the dose of 10 mg/kg bodyweight (b.w.) to each of six healthy and mastitic goats.

Experimental induction of mastitis in goats

Mastitis was induced in six clinically healthy lactating goats by Coagulase positive *Staphyloccous aureus* (procured from Indian Veterinary Research Institute, Izzatnagar, U.P., India) by method of Sar et al. (2006) with some modifications. The stock culture contained

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26×10^5 colony forming units (cfu) of *S. aureus*. 10^{-5} dilution was used for induction of mastitis. After 6 h animals showed the symptoms of inflammation (swelling and pain), fever, anorexia and agalactia. Mastitis was confirmed by the standard tests (California Mastitis Test, Somatic Cell Count, Catalase Test and Bromocresol Purple Test). On day 3rd mastitis was fully induced and pharmacokinetic study of GAT was conducted on day 4.

Collection of experimental samples

The blood samples were collected in heparinized test tubes by jugular venipuncture and milk samples were collected manually from both the quarters in sterile test tubes by hand milking at 0, 2.5, 5, 10, 15, 20, 30, 45 min and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 60 h following drug administration.

Analytical methods

The method of Santoro et al. (2006) with some modifications was used for the quantitative estimation of gatifloxacin on HPLC in plasma and milk after i.v. administration. The concentrations of gatifloxacin were determined by RP- HPLC with UV-VIS detector. The sensitivity of the method was 0.065 µg/ml and linearity was 0.999 in plasma and 0.9987 in milk are presented in Figure 1 and Figure 2.

Pharmacokinetic analysis

The pharmacokinetic parameters of GAT in goats were calculated by computer software as per methods described by Gibaldi and Weintraub, 1971; Notari, 1980; Baggot, 1977.

In vitro plasma protein binding

The plasma protein binding of GAT was determined by the "equilibrium dialysis" technique as described by Davis, (1943) and Sisodia et al. (1965).

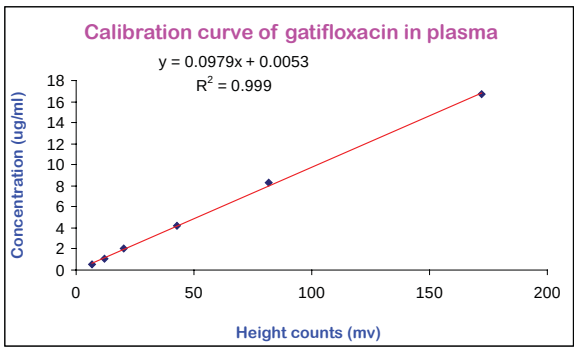

Figure 1

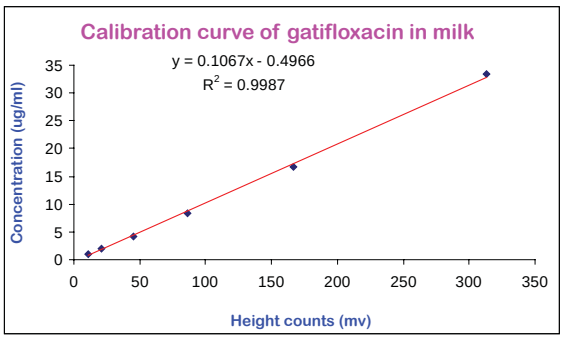

Figure 2

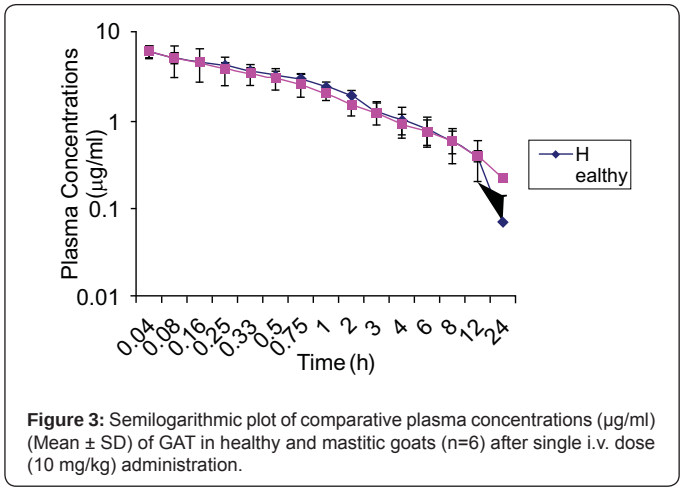

Figure 3: Semilogarithmic plot of comparative plasma concentrations (µg/ml) (Mean ± SD) of GAT in healthy and mastitic goats (n=6) after single i.v. dose (10 mg/kg) administration.

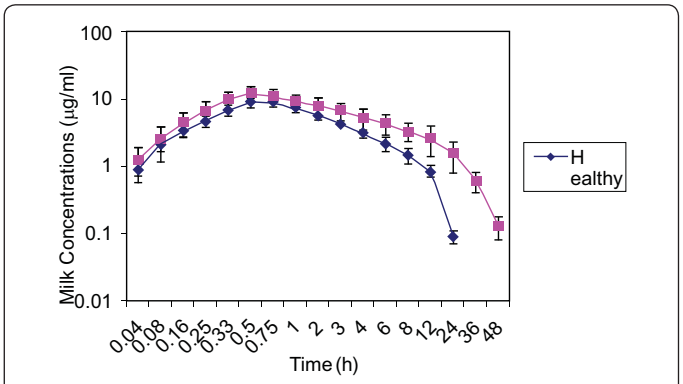

Figure 4: Semilogarithmic plot of comparative milk concentrations (µg/ml) (Mean ± SD) of GAT in healthy and mastitic goats (n=6) after single i.v. dose (10 mg/kg) administration.

Plasma standard solutions of GAT were prepared in the concentration of 6.25, 12.5, 25 and 50 µg/ml. The concentrations of drug in plasma and buffer were read with the help of HPLC and plasma protein binding of drug was calculated by the formula given by Linkenheimer et al. (1965).

$$\text{Plasma Protein binding} = \frac{\text{Conc. of drug in plasma} - \text{conc. of drug in buffer}}{\text{Conc. of drug in plasma}} \times 100$$

Stastistical method

T-test was done to see the effect of mastitis on pharmacokinetic variability of GAT according to standard method of Snedecor and Cochran, (1994).

Results

The semi-logerithmic plot of comparative gatifloxacin concentration in plasma and milk samples is presented in Figure 3 and Figure 4. In milk GAT was detected for 24 h in healthy and for 48 h in mastitic condition. Mean pharmacokinetic parameters of GAT in plasma and milk of healthy and mastitic goats are presented in Table 1. The zero-time plasma concentrations (C^0p) in healthy and mastitic goats were 7.27 ± 1.72 and 7.34 ± 1.91 µg/ml respectively. The elimination half-life ($t_{1/2}\beta$) in mastitic goats (5.82 ± 0.67 h) was significantly ($p<0.01$) higher to that in healthy goats (4.54 ± 0.75

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Kinetic parameters	Healthy	Mastitic
Plasma		
$C^{o}p$ (µg/ml)	7.27±1.72	7.34±1.91
$t_{1/2}\alpha$(h)	0.14±0.04	0.09±0.06
$t_{1/2}\beta$(h)	4.54±0.75	5.82±0.67**
Vd_{area}(L/kg)	3.92±0.69	4.76±1.03
AUC(mg/L.h)	17.40±4.51	18.03±2.06
Cl_B(ml/kg/min)	10.12±2.52	9.37±1.12
$K_{12}(h^{-1})$	4.54±2.26	5.77±2.57
$K_{21}(h^{-1})$	3.19±0.78	3.71±2.24
$K_2(h^{-1})$	0.39±0.12	0.36±0.08
MRT(h)	6.84±1.23	9.63±1.52**
T/P ratio	1.55±0.64	1.85±0.71
Milk		
Cm_{max} (µg/ml)	9.17±1.41	12.78±3.11*
$t_{1/2\beta M}$(h)	3.67±0.22	8.20±0.51**
Vd_{areaM} (L/kg)	1.32±0.17	1.29±0.43
AUC_M(mg/L.h)	40.68±4.17	104.93±36.67**
MRT(h)	5.27±0.37	12.49±0.94**
Cl_{BM}(ml/kg/min)	4.14±0.43	1.78±0.57**
T_{maxM} (h)	0.67±0.32	0.54±0.09

*P < 0.05; **P < 0.01.

C°p (µg/ml) = Zer-time plasma concentration, $t_{1/2}\alpha$(h) = Distribution plasma half-life, $t_{½}\beta$(h) = Elimination plasma half-life, Vd_{area} = Volume of distribution based on area under curve, AUC = Area under curve, Cl_B = Total body clearance rate K_{12} = Rate constant for transfer of drug from central to peripheral compartment, K_{21} = Rate constant for transfer of drug from peripheral to central compartment, K_2= elimination from central compartment, MRT = Mean residence time, AUC/MIC = Ratio between area under curve and minimum inhibitory concentration, T/P ratio = Tissue to plasma ratio. $t_{½}\beta$M = elimination half-life in milk; Vd_{areaM} = volume of distribution based on area under curve in milk; Cl_{BM}t, = total clearance in milk; Cm_{max} = maximumr milk concentration; T_{maxM} = time to peak concentration in milk.

Table 1: Plasma pharmacokinetic parameters (Mean ± S.D.) of GAT after single dose (10mg/kg) i.v. administration in healthy and mastitic goats.

h). Total body clearance (ClB) did not differ significantly in mastitic (9.37 ± 1.12 ml/kg/min) and healthy goats (10.12 ± 2.52 ml/kg/min). Apparent volume of distribution (Vd_{area}) in mastitic and healthy goats were 4.76 ± 1.03 L/kg and 3.92 ± 0.69 L/kg respectively. The ratio of K_{12}/K_{21} in mastitic goats (1.75 ± 0.67) was slightly higher than healthy (1.31 ± 0.70). Plasma protein binding of GAT was found to be 25 %.

Maximum milk concentration (Cm_{max}) in mastitic goats (12.78 ± 3.11 µg/ml) was significantly ($p<0.05$) higher than healthy goats (9.17 ± 1.41 µg/ml). Milk half-life ($t_{½\beta M}$) in mastitic goats (8.20 ± 0.51 h) was significantly ($p<0.01$) higher than healthy (3.67 ± 0.22 h). The value of AUC in mastitic milk (104.93 ± 36.67 mg/L.h) was significantly ($p<0.01$) higher than healthy (40.68 ± 4.17 mg/L.h). Time for attainment of maximum milk concentration ($t_{max\,M}$) in healthy goats was 0.67 ± 0.32 h, whereas in mastitic goats it was obtained earlier (0.54 ± 0.09 h).

Discussion

The semi-logarithmic plot of plasma levels time profile of GAT evident bi-exponential decay curve and the pharmacokinetic parameters were described based on two compartment open model in healthy and mastitic goats (Baggot, 1977). The plasma half-life ($t_{½}\beta$) of GAT was significantly ($p<0.01$) longer in case of mastitic goats as compared to healthy, decreased ClB in mastitic goats also supports the finding. The K_{12}/K_{21} ratio obtained in this study indicated a faster drug transfer from central to peripheral compartment than from peripheral to central compartment. The plasma protein binding of GAT was found to be 25%. In humans also the serum protein binding is approximately 20% (Grasela, 2001). This lower value of plasma protein binding is suggesting that the drug in not remaining in the vascular compartment only, rather it is being distributed widely. It is further supported by slight increase in the value of Vd_{area} in mastitic goats. The T/P ratio in mastitic goats was higher than healthy, exhibiting more concentration of drug in tissues than plasma, which would be of significance while considering the case of mastitis where there is need of concentration of more drug in milk than plasma.

Milk half-life ($t_{½\beta M}$) of GAT was also found to be significantly longer in mastitic goats as compared to healthy. However, GAT was maintained for a longer period in milk than plasma of mastitic goats. Similar results were also reported in subjects suffering with pneumococcal meningitis, $t_{½\beta}$ of GAT in CSF (3.8 to 5.6 h) was found higher as compared to blood (2.7 to 3.2 h) (Lutsar et al., 1998). The $t_{½\beta}$ of GAT in plasma (6.8 h) has also been found to be lower as compared to that in inflammatory fluid (7.2 h) (Wise et al., 1999). The Cm_{max} of GAT in case of mastitic goats was significantly higher than healthy goats. The various solute transport and secretion processes involved in milk production offer pathways for the movement of drug molecules from plasma to milk (McManaman and Neville, 2003). It may be mentioned that un-ionized drug molecule is easily diffusible across the membranes and the extent of ionization depends upon pH of the fluids in the compartments. GAT has two Pka values, Pka1-6.0 and Pka_2-9.2. The plasma pH is relatively constant (7.4) because of its buffering system. Milk pH, however, is more variable and increase markedly in mastitic conditions. The results obtained in this experiment strengthen the argument that GAT passed from blood to milk via non-ionic diffusion. Increased permeability of the mammary epithelial cells under the effect of several chemical mediators (Zhao and Lacasse, 2007) may contribute to some extent, in the increased milk concentration of GAT in mastitic goats. There is an additional fact that polymorphonuclear neutrophils uptake GAT, like other fluoroquinolones. The active uptake of GAT by the neutrophils is beneficial from treatment point of view. The $t_{½}$ milk/$t_{½\,plasma}$ ratio (1.41) also indicated drug persistence in mastitic milk for longer period than in plasma. Reports are available that ibafloxacin penetrated poorly from blood into milk after i.v. administration in goats and persisted for short time in milk than in plasma ($t_{½}$ milk/$t_{½}$ plasma < 1) (Marin et al., 2007a). It may be mentioned that quinolones are concentration-dependent killers and for effective systemic treatment of mastitis drug should extensively penetrate the inflamed mammary gland. In this experiment it was found that the AUCmilk/AUCplasma ratio in mastitic goats was 5.82 which indicated that the drug penetrated milk extensively. For orbifloxacin administered i.v. AUC_{milk}/AUC_{plasma} ratio was 1.02 (Marin et al., 2007b). The minimum inhibitory concentration of GAT for *S. aureus* has been reported to be 0.1 µg/ml (Tsurumaki et al., 2000; Boubakar et al., 2006). AUC/MIC is one of the most important efficacy predictor with the rate of clinical cure being >80%, The AUC/MIC ratio of GAT in plasma and milk of mastitic goats were 180 and 1049 respectively, which indicated good therapeutic efficacy.

On the basis of above observed findings, it can be said that GAT showed excellent milk penetration, maintenance of higher concentration for a longer time period and slower elimination from mastitic goats. Thus, it would be helpful for treating mastitis in goats caused by *S. aureus*.

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