PENETRATION OF DANOFLOXACIN INTO TISSUES OF THE RESPIRATORY AND GASTROINTESTINAL TRACT IN CALVES

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INTRODUCTION

Danofloxacin* (CP-76,136) is a new fluoroquinolone which possesses high *in vitro* activity against the major bacterial pathogens in farm animals (1). The spectrum and potency of this drug indicate potential utility in the treatment of a broad range of infections, including pneumonia and enteritis. To guide effective therapy, this study focuses on the pharmacokinetics of danofloxacin in calves and the penetration of the drug into tissues of the respiratory and gastrointestinal tract.

MATERIALS AND METHODS

Experiments were performed in two groups of Danish-Holstein calves 4 to 6 weeks old and weighing from 47 to 68 kg. The calves were fed 3 L of milk replacer twice daily and provided *ad libitum* concentrate, hay and water.

Group A. The pharmacokinetics of danofloxacin were studied in four calves after IV administration of 1.3 ± 0.05 mg/kg. Blood samples were collected at predetermined times up to 24 hours after dosing. Nasal and bronchial secretion specimens were obtained at 1, 2, 4, 8, 12 and 24 hours after dosing. Secretions were collected by a tampon method described by Raun and Friis. (2).

Group B. The tissue distribution of danofloxacin was determined in 12 calves after IV administration of 1.3 ± 0.04 mg/kg. Blood samples were collected as in Group-A calves and bronchial and nasal secretion specimens were harvested immediately before euthanasia. Calves were killed by use of a bolt pistol; two each at 1, 2, 4, 8, 12 and 24 hours after dosing. Lungs and gastrointestinal tract were removed intact and specimens of lung, duodenum, jejunum, ileum, colon and mesenterial lymph nodes were collected. Bronchial mucosa was sampled by dissection of the large bronchi, whereas mucosa of the intestine were harvested after freezing pieces of tissue to -20° C followed by thawing to a temperature of 0° C.

Danofloxacin was measured by high-pressure liquid chromatography (HPLC) The detection limit was 10 ng/ml for plasma and secretions and 50 ng/g for tissues.

RESULTS

Group A. Danofloxacin disposition was best described by an open 3-compartment model. The drug was distributed rapidly in the body with an initial distribution half-life ($t_{½\lambda 1}$) of 0.1 hour and

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a second ($t_{½\lambda 2}$) of 1.6 hour. The elimination half-life ($t_{½\lambda 3}$) averaged 7.4 hours and the body clearance (Cl_{tot}) 0.55 L/kg/hour (Table 1). The volume of distribution (V_{ss}) was 4.3 L/kg, indicating that the drug is concentrated within tissues.

Danofloxacin concentration in nasal and bronchial secretions peaked at the first sampling point 1 hour after dosing, being 0.16 ± 0.01 and 0.27 ± 0.16 µg/ml, respectively. After maximum the curves declined in parallel with the plasma curve resulting in a secretion to plasma ratio of 0.48 in the nose and 0.97 in the bronchi. Area under the curve (AUC) in bronchial secretions comprised 85% of the AUC in plasma with no significant difference between the areas (paired t-test). In nasal secretions AUC made up 42% of the plasma AUC.

Table 1

Selected pharmacokinetic values after IV administration of 1.3 mg/kg.

		Group A Means ± SD	Group B Pooled data
n		4	12
dose	mg/kg	1.34 ± 0.05	1.33 ±0.04
$t_{½\lambda 3}$	hour	7.4 ± 1.8	6.3
Cl_{tot}	L/hour/kg	0.55 ± 0.20	0.48
V_{ss}	L/kg	4.3 ± 0.8	3.8

Group B. The pharmacokinetic values of pooled data from Group-B calves corresponded well with those from Group-A calves (Table 1). As in Group A, danofloxacin concentration in nasal and bronchial secretions parallelled the plasma concentration (Table 2). In lung tissue and bronchial mucosa peak concentrations were reached at 1 hour being approximately 5 and 3 times higher than the plasma level. Similarly mean tissue to plasma AUC ratio was approximately 5 for lung tissue and 3 for bronchial mucosa.

Table 2

Danofloxacin concentrations in the respiratory tract after IV administration of 1.3 mg/kg (Group B). Average of 2 animals at each time.

TIME hours	PLASMA µg/ml	NASAL SECRETIONS µg/ml	BRONCHIAL SECRETIONS µg/ml	BRONCHIAL MUCOSA µg/g	LUNG TISSUE µg/g
1	0.42	0.23	0.57	1.36	2.24
2	0.29	0.14	0.40	0.87	1.44
4	0.20	0.10	0.23	0.62	0.77
8	0.09	0.04	0.09	0.22	0.35
12	0.07	0.03	0.07	0.16	0.21
24	0.02	0.01	0.02	0.05	0.07

In the gastrointestinal tract peak concentration of danofloxacin was likewise reached 1 hour after drug administration in all locations except colon (Table 3). In the latter danofloxacin concentration peaked at 4 h. Peak tissue to plasma concentration ratio was approximately 4 in lymph nodes, and 4.5, 5.0, 5.5 and 3.5 in duodenum, jejunum, ileum and colon, respectively. The tissue to plasma AUC ratio was 3.9 for lymph nodes and 3.5, 3.5, 4.4 and 7.6 for duodenum, jejunum, ileum and colon, respectively. Danofloxacin concentration in the intestinal mucosa was almost identical to the level found in total tissue for the various segments (Table 4).

Table 3

Danofloxacin concentrations in total tissue of the gastrointestinal tract after IV administration of 1.3 mg/kg (Group B). Average of two animals at each time.

TIME	DUODENUM	JEJUNUM	ILEUM	COLON	LYMPH NODES
hours	µg/g	µg/g	µg/g	µg/g	µg/g
1	1.91	2.13	2.33	1.22	1.74
2	1.09	1.16	1.55	1.02	1.30
4	0.75	0.85	0.87	1.81	0.74
8	0.32	0.27	0.36	0.75	0.29
12	0.18	0.20	0.33	0.93	0.31
24	0.07	0.05	0.07	0.13	0.08

Table 4

Danofloxacin concentrations in mucosa of the gastrointestinal tract after IV administration of 1.3 mg/kg (Group B). Average of two animals at each time.

TIME	DUODENUM	JEJUNUM	ILEUM	COLON
hours	µg/g	µg/g	µg/g	µg/g
1	2.25	1.74	1.98	1.31
2	1.17	0.88	1.28	1.45
4	0.68	0.69	0.62	1.98
8	0.23	0.21	0.32	1.16
12	0.18	0.15	0.33	1.14
24	0.06	0.03	0.05	0.14

DISCUSSION AND CONCLUSION

Danofloxacin was rapidly and widely distributed in the body, whereas the elimination was rather slow. In the respiratory tract danofloxacin was accumulated more extensively in total lung tissue than in bronchial mucosa. This variation may reflect the different composition of the tissues. Lung tissue comprises several compartments including phagocytes, which accumulate fluoroquinolones up to 10 times concentration in extracellular

fluid (3). Danofloxacin concentration in bronchial secretions was similar to that in plasma.

In acute pneumonia the principal localization of pathogens is in bronchial secretions and epithelium. Therefore, concentrations at these sites appear to be more relevant for evaluating the therapeutic potential than concentration in total lung tissue. In surveys (1,4) the MIC_{90} of danofloxacin against the common respiratory pathogens - *Pasteurella* spp. and *Haemphilus somnus* was found to be below 0.1 µg/ml. Accordingly, IV administration of 1.3 mg danofloxacin/kg maintains active concentrations above MIC_{90} for up to 12 hours in bronchial mucosa and up to 8 hours in bronchial secretions.

Danofloxacin concentration in the mucosa of various segments of the gastrointestinal tract was almost identical to findings in total tissue. This coincides with findings for other fluoroquinolones which indicate high accumulation in mucosa and high excretion into ingesta (4, 6). Since MIC_{90} of danofloxacin against the common enteric pathogens - *E.coli* and *Salmonella* spp. was found to be below 0.03 µg/ml (1), IV administration of 1.3 mg/kg produces active drug concentration in the entire intestine for more than 24 hours.

SUMMARY

The study focused on the pharmacokinetic profile of danofloxacin, a new fluoroquinolone, and its penetration into the tissues of the respiratory and gastro-intestinal tract of calves. Danofloxacin was administered at a dosage of 1.3 mg/kg body weight. Blood and tissue samples were collected at predetermined times up to 24 hours after dosing. Assays were performed using HPLC. Danofloxacin distribution was described by an open 3-compartment model. Mean elimination half-life was 7.4 h, total body clearance 0.55 L/h/kg and steady state volume of distribution 4.3 L/kg. Peak concentrations in lung, bronchial mucosa and secretions were achieved 1 h after drug administration. Danofloxacin accumulated extensively in total lung tissue and in bronchial mucosa, with peak concentrations 5 and 3 times higher than plasma level. Danofloxacin concentration in bronchial secretions was similar to that in plasma. In the gastrointestinal tract, peak concentrations were reached 1 hour after administration in all locations sampled, except colon, where concentration peaked at 4 hours. Danofloxacin concentration in the intestinal mucosa was almost identical to concentrations found in total intestinal tissue. Peak concentrations in intestinal tissues varied between 4 and 5 times the plasma concentrations.

RESUMEN

El estudio estuvo enfocado hacia el perfil farmacocinetico de danofloxacin, una nueva fluoroquinolona, y a su penetracion en los tejidos del tracto respiratorio y del tracto gastrointestinal en terneros. Danofloxacin fue administrado a una dosis de 1.3 mg/Kg de peso. Se colectaron muestras de sangre y de tejidos a intervalos de tiempo predeterminados por hasta 24 horas post dosificacion. Los ensayos se realizaron mediante cromatografia liquida de alta

presion (HPLC).La distribucion de danofloxacin fue descrita mediante un modelo abierto de 3 compartimentos. El promedio de vida media de eliminacion fue 7.4 horas, la eliminacion corporal total 0.55 L/h/Kg y el volumen de distribucion en la fase de equilibrio dinamico 4.3 L/Kg. Las concentraciones pico en pulmon, mucosa bronquial y secreciones fueron alcanzadas 1 hora despues de la administracion de la droga. Danofloxacin se acumulo extensivamente en la totalidad de los tejidos pulmonares y en la mucosa bronquial, con concentraciones pico 5 y 3 veces mas altas que el nivel en plasma. La concentracion de danofloxacin en las secreciones bronquiales fue similar a la concentracion plasmatica. En el tracto gastrointestinal, las concentraciones pico se alcanzaron 1 hora despues de la administracion en todas las porciones muestreads, excepto en el colon, donde la concentracion pico fue alcanzada despues de 4 horas. La concentracion de danofloxacin en la mucosa intestinal fue casi identica a las concentraciones encontradas en tejido intestinal completo. Las concentraciones pico en los tejidos intestinales variaron entre 4 y 5 veces las concentraciones en el plasma.

RÉSUMÉ

Cette étude traite du profil pharmacocinétique de la danofloxacine, une nouvelle fluoroquinolone, et de sa pénétration dans les tissus du tractus respiratoire et gastro-intestinal des veaux. La danofloxacine fût administrée à une dose de 1,3 mg/kg de poids vif. Des prélèvements de sang et de tissu furent effectués à des intervals de temps prédéterminés, jusqu'à 24 heures après injection. Ces échantillons furent analysés par HPLC. La distribution de la danofloxacine fût décrite suivant un modèle ouvert à 3 compartiments. Ces analyses montrèrent une demi-vie d'élimination de la molécule de 7,4 heures, une clairance de 0,55 l/h/kg et un volume apparent de distribution de 4,3 l/kg. Des concentrations maximales dans les poumons, dans la muqueuse et les sécretions bronchiques furent atteintes 1 heure après l'injection de la molécule. La danofloxacine se concentra dans tout le tissu pulmonaire ainsi que dans la muqueuse bronchique avec des pics de concentrations 5 à 3 fois supérieur à sa concentration plasmatique. La concentration de danofloxacine dans les sécretions bronchiques fût similaire à celle observée dans le plasma. Dans le tractus gastro-intestinal, des pics de concentration furent atteints 1 heure après l'injection de la molécule, dans toutes les parties prélevées, sauf dans le colon où le pic fût atteint après 4 heures. La concentration de la danofloxacine dans la muqueuse de l'intestin fût similaire à celle dans l'intestin lui-même. Les pics de concentration de la danofloxacine dans les tissus intestinaux atteignirent des niveaux compris entre 4 et 5 fois la concentration plasmatique.

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