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Research Paper

The Pharmacokinetics of the Weakly Protein-Bound Anionic Compound Diatrizoate in Serum and Synovial Fluid of the Horse

Anna Buus Frost,[1] Frank Larsen,[2] Susan Weng Larsen,[1] Jesper Østergaard,[1] Maj Halling Thomsen,[3] Stefan Stürup,[1] Pia Haubro Andersen,[3] and Claus Larsen[1,4]

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Purpose. To establish a pharmacokinetic model for the model drug, sodium diatrizoate (DTZ), allowing joint disappearance kinetics to be estimated from serum appearance kinetics following intra-articular administration, and to calculate the relative joint exposure after intravenous and intra-articular DTZ administration ($F_{iv/IA}$).
Methods. Each of five horses received an aqueous solution of 3.9 mg/kg sodium diatrizoate both intravenously and intra-articularly separated by a one-week wash out period. Serum and synovial samples were collected over 7 h and analyzed for content of model compound using inductively coupled plasma mass spectrometry.
Results. Differential equations were used for describing the transport of DTZ between the joint and the central compartment. The three-compartment lag-time model obtained demonstrates that the rate of drug appearance in the systemic circulation equals the rate of disappearance from the joint compartment. Following intravenous and intra-articular administration, an average $F_{iv/IA}$ of 0.04% ($n=4$) was calculated based on the synovial fluid profiles of DTZ.
Conclusions. This study implies that aspects of the intra-articular fate of DTZ can be obtained from serum data in case synovial fluid samplings are limited, for various possible reasons. The low $F_{iv/IA}$ may stimulate future research in the field of intra-articular administration of anti-osteoarthritic drugs.

KEY WORDS: disappearance kinetics; intra-articular administration; pharmacokinetics.

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis in humans with an estimated prevalence of about 7 % (1). This arthritic disorder is characterized by a slow, progressive breakdown of the articular cartilage and affects, most often, one or only a few joints. No cure is available, and current pharmacological interventions, mainly addressing pain, are only moderately effective (2). Oral treatment options embrace non-steroidal anti-inflammatory drugs (NSAIDs) and other analgesics (3). Also, intra-articular (IA) therapies are available, including long-acting glucocorticoid suspensions and hyaluronic acid products (4). After oral dosing, only a very small fraction of the administered dose enters a specific disease-affected joint (5). Compared to oral dosing, direct injection of the drug into such a joint allows for a significant dose reduction and thereby minimizes the risk of the known systemic adverse effects. Recent identification of novel potential drug targets within the synovial space may give optimism to the development of new disease-modifying therapies in the area of OA (6,7). Among such targets are matrix metalloproteinases (MMPs), which participate in the destruction of the extracellular matrix of the articular cartilage (6,8). Orally administered MMP inhibitors have been tested in clinical trials (6). So far, these drug candidates have failed due to adverse effects. Therefore, IA administration may be an alternative not least for novel anti-arthritic drugs which are intolerable when administered systemically (9). In general, drugs dissolved in the synovial fluid (SF) exhibit short residence times within the joint cavity with disappearance half-lives of about 0.1–6 h (5). Hence, future disease-modifying osteoarthritic drugs (DMOADs) may require IA injection in the form of a suitable depot formulation to maintain therapeutic drug concentrations within the joint cavity for as long as possible (10).

[1] Department of Pharmaceutics and Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen, Universitetsparken 2, DK-2100, Copenhagen Ø, Denmark.
[2] Clinical Pharmacology and Pharmacokinetics, H. Lundbeck A/S, Ottiliavej 9, DK-2500, Valby, Denmark.
[3] Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Højbakkegård Allé 5, DK-2630, Taastrup, Denmark.
[4] To whom correspondence should be addressed. (e-mail: csl@farma.ku.dk)

ABBREVIATIONS: AIC, Akaike information criterion; AUC, Area under the curve; C_0, Concentration at time zero; C_{max}, Maximum concentration; DMOAD, Disease modifying osteoarthritic drug; DTZ, Sodium diatrizoate; $F_{iv/IA}$, The relative joint exposure after iv and IA drug administration; IA, Intra-articular; ICP-MS, Inductively coupled plasma mass spectrometry; iv, Intravenous; MMP, Matrix metalloproteinases; NSAID, Non-steroidal anti-inflammatory drug; PBS, Phosphate buffer solution pH 7.4; RCF, Relative centrifugal force; t_{max}, Time for maximum concentration.

Aqueous suspensions of poorly soluble steroids remain the only world-wide, commercially available depot formulation for IA injection. Such formulations have been used in the clinic for more than three decades (11,12). Despite that the performance of a variety of depot principles has been investigated *in vivo*, the IA fate of drugs released from such sustained release injectables is far from fully understood. Most studies have been carried out in smaller animals and most often in the rabbit (5). The adequacy of employed injection methodologies to minimize trauma, tissue damage and extra-articular leakage (13,14) has not been fully addressed in these studies. Access to a suitable animal model constitutes a key prerequisite in the investigation/optimization of the release characteristics of a novel IA depot formulation. Preferably the test animal should (i) be easy to handle, (ii) be fully conscious throughout the entire handling period, and (iii) possess sufficiently large joints allowing multiple SF sampling after administration of a drug solution as well as reproducible depot instillation. The horse exhibits these attributes and was chosen as animal model in the present study.

Both the rate of release from a given depot and the transport rate of the drug solute out of the synovial space influence the IA drug concentration *versus* time profile. As a contribution to the understanding of drug disappearance kinetics after IA depot injection, the present study was undertaken to determine the pharmacokinetics of a highly hydrophilic model drug compound (sodium diatrizoate (DTZ) (15)) injected into the equine radiocarpal joint in the form of an aqueous solution. DTZ has a carboxylic acid group (pK_a 3.4, (16)). Hence DTZ will be fully ionized in both serum and synovial fluid. No metabolites of DTZ are known, and, therefore, this compound is considered metabolically stable (17). The objective was to develop a pharmacokinetic model for DTZ allowing joint disappearance kinetics to be estimated from serum appearance kinetics. Further, the study was designed to enable calculation of the relative joint exposure after intravenous (iv) and IA drug administration ($F_{iv/IA}$), a parameter for which only few estimates are available in the literature (18).

MATERIALS AND METHODS

Materials

Sodium diatrizoate (DTZ - Fig. 1) was purchased from Sigma-Aldrich (St. Louis, USA). Other reagents, buffer substances and solvents were of analytical or reagent grade. Deionised or higher quality water was used throughout the study. Heparin "Leo" 5,000 iu/ml was obtained from Leo Pharma (Ballerup, Denmark). Sodium Chloride "SAD," 9 mg/ml, Bupivacaine "SAD" 5 mg/ml and Lidocaine "SAD" 20 mg/ml were purchased from Amgross I/S (Copenhagen, Denmark).

DTZ Solution for Injection

DTZ was dissolved in distilled water to obtain a concentration of 280 mg carboxylate anion/ml. The slightly alkaline solution was prepared under aseptic conditions, filtered (0.22 μm) and autoclaved (121°C, 15 min). DTZ in phosphate buffer solution (PBS) (67 mM, pH 7.4) is stable at 37°C for at least 14 days in accordance with the observation that DTZ exhibits high stability in neutral aqueous solution (19).

Animals

Five adult horses (crossbreed) were used in the study (331–534 kg). The horses were healthy and free from evidence of joint disease based on a thorough clinical examination, haematology, serum biochemistry and SF analysis. During the entire study period, the horses were housed in 3×4 m stalls in a stable with a constant temperature of 13±1°C at facilities of the University of Copenhagen. Horses were fed a commercial grain mixture twice a day and had free access to water and hay. The experimental protocol was pre-approved by the Danish Animal Experimentation Board, and all procedures were carried out according to the Danish Animal Testing Act.

Experimental Design

Each horse was in random order subjected to an iv and an IA administration separated by a one-week wash-out period. The horses were dosed 3.9 mg DTZ/kg in both iv and IA studies. For the iv administration, the solution of DTZ was injected into the jugular vein (bolus injection), and for the IA administration, the solution of DTZ was injected into the radiocarpal joint of the horse (bolus injection). All volumes administered were under 10 ml.

Sampling

Blood samples of 10 ml were collected from a jugular vein catheter, at −5, 5, 10, 20, 30, 40, 60, 75, 90, 120, 150, 180, 240, 300, 360 and 420 min after administration. All blood samples were allowed to clot at room temperature, and serum was isolated in evacuated tubes (BD Vacutainer®, Franklin Lake, USA). SF samples of 0.4 ml were withdrawn from the radiocarpal joint by repeated arthrocentesis using standard aseptic techniques at −5, 10, 20, 30, 60, 90, 120, 180, 240, 300 and 420 min after administration. All synovial samples were stabilized in EDTA-evacuated tubes (BD Vacutainer®). Serum and SF samples were frozen at −20°C until analysis.

Sample Preparation

SF and serum samples were deprotenized by addition of 3 volumes of methanol and vortexed. After centrifugation at 14.000 relative centrifugal force (rcf) for 15 min, the supernatant was diluted (minimum 10 times) with 7.5 % (v/v) methanol in phosphate buffer (10 mM, pH 7.4). The diluted samples were ready for analysis.

Analysis

Concentrations of DTZ in serum and SF samples were quantified by measuring m/z 127 using an inductively coupled plasma mass spectrometry (ICP-MS) method based on the fact that atomization of DTZ liberates 3 iodine atoms (Fig. 1). The ICP-MS instrument applied was a PE Sciex Elan DRC-e (Perkin Elmer, Norwalk, CT, USA) equipped

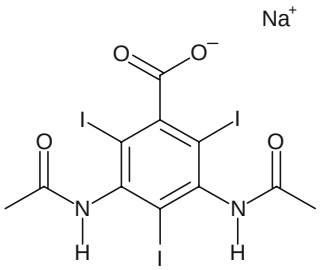

Fig. 1. Sodium diatrizoate (DTZ).

with a PEEK Mira Mist parallel path nebulizer (Burgener Research Inc., Mississauga, Ontario, Canada) and a water-cooled cyclonic spraychamber operated at 4°C (Glass Expansion, Melbourne, Australia). The sample uptake rate was 200 µl/min. ICP-MS sampler and skimmer cones were made of platinum. The plasma and auxiliary gas flow rates were 15 l/min and 1.2 l/min, respectively. The nebulizer gas flow, lens voltage and RF power settings were optimized daily for maximum sensitivity using a solution of 6 µg/l DTZ in 7.5% (v/v) methanol and phosphate buffer (10 mM, pH 7.4). A count rate of approximately 10,000 cps was normally achieved. Normal setting for nebuliser gas flow, lens voltage and RF power were 1.2 l/min, 15 V and 1,050 W, respectively. The data acquisition settings were 200 msec dwell time, 1 sweep per reading, 40 readings per replicate, and 5 replicates producing a total analysis time of 40 sec per sample. Between samples, the instrument was washed for 90 sec with 7.5% (v/v) methanol in deionised water. Quantification was performed using a matrix-matched external standard curve. Standards ranged from 1.5 to 500 µg/l of DTZ. Standards were made by adding DTZ to blank SF or serum samples taken before administration of DTZ and treated as described for samples in the previous section. Repeatability was acceptable (RSD=1.7 %, n=6 at concentrations close to limit of quantification (LOQ)), and the LOQ was 1 µg/l calculated as 10 times the standard deviation of 6 injections of a 6 µg/l DTZ solution in PBS. Endogenous iodine in serum and SF was found to be low and assumed to be constant over the duration of each experiment (7 h) (e.g. H5, prepared serum sample, RSD=8 % (n=4), below 3 µg/l). The samples from each horse were corrected for the small contribution of endogenous iodine. In all cases, DTZ concentrations exceeded that of endogenous iodine by a minimum factor of 4. By far, the majority of the diluted serum and SF samples contained above 70 µg/l.

Pharmacokinetic Analysis

The observed maximum concentration of DTZ (C_{max}) and the time to maximum concentration (t_{max}) were determined. The terminal half-lives ($t_{½}$) using the first-order rate constant associated with the terminal (log-linear) portion of the curves were calculated. The area under the curve (AUC) was calculated by the linear trapezoidal rule using standard non-compartmental analysis. The $AUC_{0\text{-}t}$ was calculated, and the residual area ($AUC_{t\text{-}\infty}$) was determined as the ratio of the observed concentration at the last time point to the corresponding terminal rate constant. $AUC_{0\text{-}\infty}$ was expressed as the sum of $AUC_{0\text{-}t}$ and $AUC_{t\text{-}\infty}$. The relative joint exposure after iv and IA drug administration ($F_{iv/IA}$) was calculated using Eq. 1:

$$F_{iv/IA} = \frac{Dose_{IA} \cdot AUC_{iv,\,0-\infty}}{Dose_{iv} \cdot AUC_{IA}} \cdot 100 \tag{1}$$

where the AUC_{iv} is the area under the SF curve after iv administration, AUC_{IA} is the area under the SF curve after IA administration, $Dose_{IA}$ is the dose-administered IA and $Dose_{iv}$ is the dose-administered iv. In this experimental setup, the dose-administered iv and the IA were the same.

In the compartmental modeling, DTZ SF and serum concentrations were analyzed simultaneously, and the differential equations were parameterized in terms of intercompartmental rate constants (k_e, k_{cj}, k_{jc}, k_{cp}, k_{pc}) (Fig. 2). The general differential equation system associated with the transport processes of each administration route depicted in Fig. 2 was as follows:

$$\frac{dA_c}{dt} = k_{jc}A_j - k_eA_c - k_{cj}A_c + k_{pc}A_p - k_{pc}A_c \tag{2}$$

$$\frac{dA_j}{dt} = k_{cj}A_c - k_{jc}A_j \tag{3}$$

$$\frac{dA_p}{dt} = k_{cp}A_c - k_{pc}A_p \tag{4}$$

where A_c and A_p represent the drug amounts in the central and peripheral compartments, and A_j is the amount in the joint compartment (Fig. 2). The estimated concentrations in the central and the joint compartment are calculated as

$$C_j = \frac{A_j}{V_{joint}} \tag{5}$$

$$C_c = \frac{A_c}{V_{central}} \tag{6}$$

where V_{joint} and $V_{central}$ are the distribution volumes of the joint and the central compartment, respectively. The clearance of DTZ from the central compartment ($CL_{central}$) was calculated according to Eq. 7:

$$CL_{central} = k_e \cdot V_{central} \tag{7}$$

where k_e is the first-order elimination rate constant. The clearance of DTZ into the joint (CL_{cj}) and the clearance out of the joint (CL_{jc}) were calculated using Eq. 7 and the respective rate constants and distribution volumes. The software WinNonlin® (version 5.2, Pharsight Corporation, Mountain View, CA, USA) was used for the pharmacokinetic modeling, and the Nelder-Mead algorithm was used. Model selection was based on the Akaike information criterion (20). Weighting and goodness-of-fit were based on visual inspection of the data plotted in a semi-logarithmic graph. The data were weighted according to $1/y_j^2$ where y_j is the predicted value of the j^{th} observation.

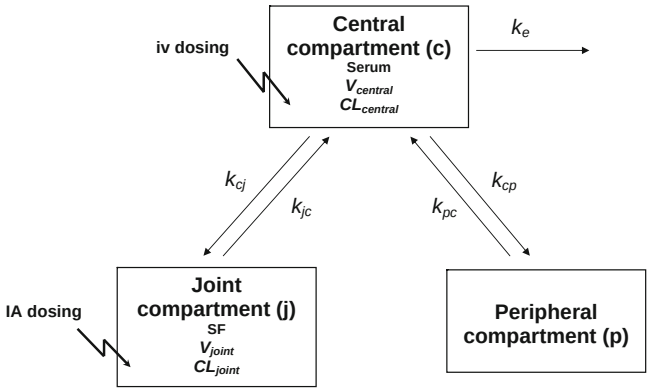

Fig. 2. Three-compartment model applied in the pharmacokinetic analysis of serum and SF data after iv and IA administration, respectively. k_{xy} are first-order rate constants.

RESULTS

Serum and SF concentration *versus* time profiles for DTZ after both IA and iv administration of 3.9 mg/kg to horses (n=5) are presented in Fig. 3 (raw data tabulated in Supplementary Material). The pharmacokinetic model used for describing the SF and serum concentration profiles after IA and iv injection of DTZ, respectively, is depicted in Fig. 2. A representative example of the comparison between the predicted and the observed DTZ concentrations *versus* time profiles (H2) is shown in Fig. 4. Table I contains a compilation of selected pharmacokinetic parameters, and in Table II the micro-constants obtained from the pharmacokinetic modeling procedure are presented.

After IA administration, both the serum and the SF concentration *versus* time profiles of one of the horses (H1)

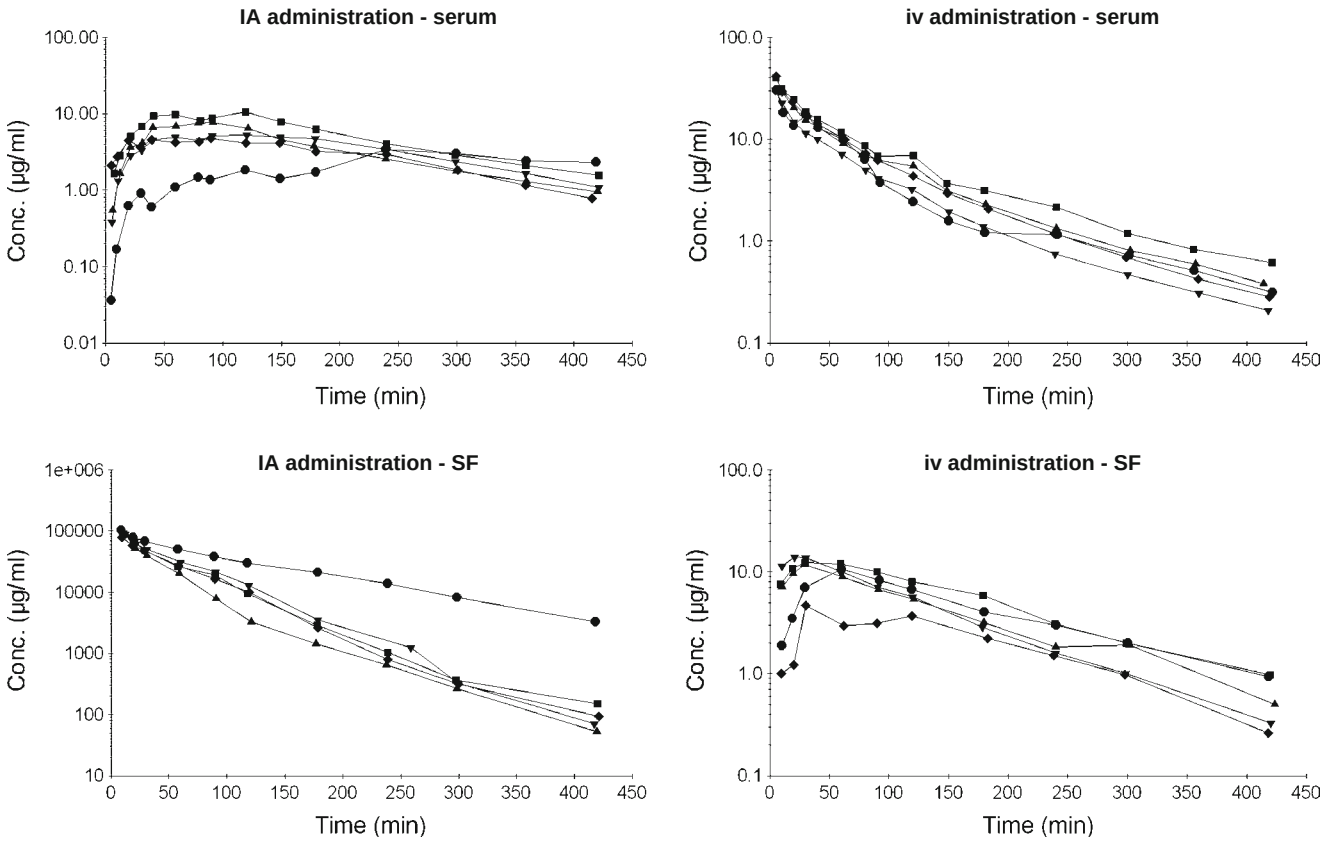

Fig. 3. DTZ concentrations (µg/ml) after dosing 3.9 mg DTZ/kg to horses (n=5, ●=H1, ■=H2, ◆=H3, ▲=H4, ▼=H5).

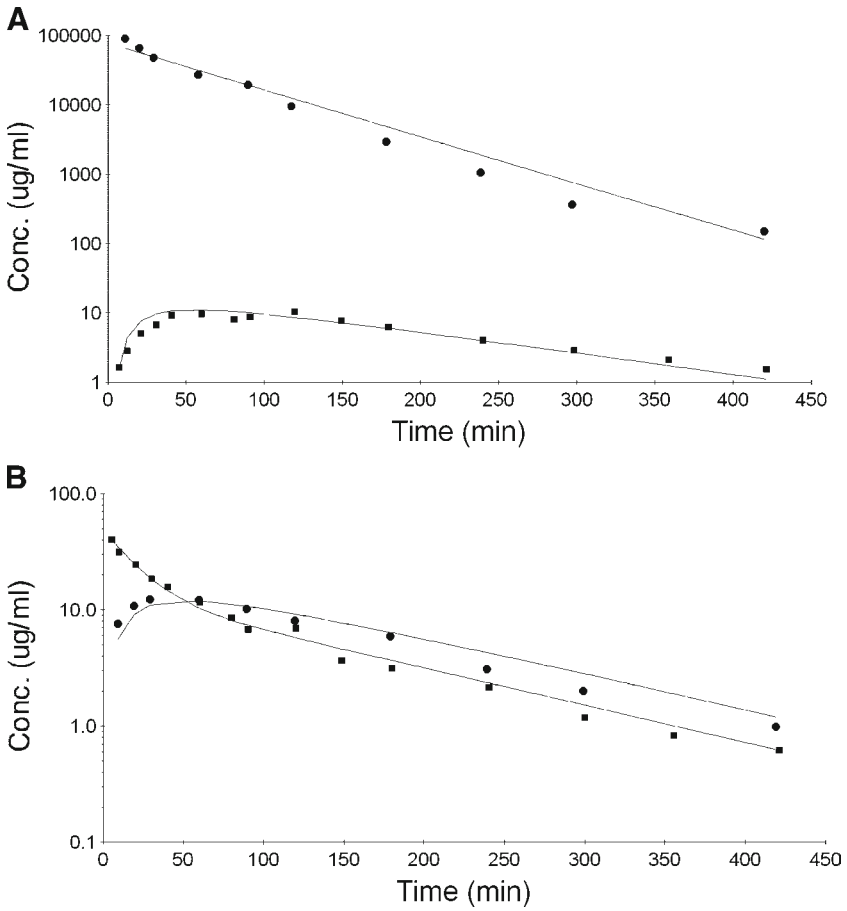

Fig. 4. Predicted (−) and observed DTZ concentrations (dosing 3.9 mg/kg) for a representative horse (H2) in **A** SF (●) and serum (■) after IA administration **B** SF (●) and serum (■) after iv administration.

were observed to differ from those obtained from the remaining four horses (Fig. 3). There was nothing in the experimental setup or any deviating behavior during the performance of the study that could explain the deviating profile obtained for H1. Compared to the other 4 horses, the disappearance of DTZ from the joint proceeded significantly slower for H1 (Fig. 3, Table I). This behavior resulted in a delayed appearance of DTZ in serum (Fig. 3). Therefore, as regards this route of administration calculations for H1 have been omitted.

The maximal DTZ concentration in serum after IA administration was 4.8–10.5 µg/ml, t_{max} values were 90–120 min and the apparent half-lives were 91–136 min (n=4) (Table I). After IA administration, the half-lives in SF ($t_{½,\ joint}$) were 39–50 min (Table I). The median rate constant associated with the disappearance of DTZ in the serum (k_e) was 0.0199 min^{-1} (range 0.0183–0.0273 min^{-1}) (Table II). A two-compartment model was first applied to the data, but the three-compartment model (Fig. 2) had the lowest AIC value and the lowest %RSDs. Furthermore, a lag-time was introduced to the three-compartment model, and the AIC value decreased even more (from joint to central compartment, range 1–5 min). The model used for description of DTZ pharmacokinetics for H2-H5 (Fig. 2) was, however, not applicable to H1. Values of the rate constant for the disappearance of DTZ from the SF after IA administration (k_{jc} in Fig. 2) were in the range 0.0156–0.0180 min^{-1} (Table II).

After iv administration, the mean serum half-life ($t_{½,\ central}$) was 98 min (RSD 3%)(Table I). Maximum SF concentration after iv administration was found in the range 4.7–14.0 µg/ml, whereas the t_{max} and the apparent $t_{½}$ ranges were 21–61 min and 78–109 min, respectively (Table I). Almost identical ranges for the rate constants k_{cp} and k_{pc} related to the distribution in and out of the peripheral compartment were observed (Table II). Values of the rate constant k_{cj} are listed in Table II. The mean clearance $CL_{central}$ was 2.0 ml/min/kg. The ratio CL_{cj}/CL_{jc} has been calculated, revealing a median of 0.98 (range 0.75 – 2.5). A clearance ratio close to unity would be anticipated for drugs with no protein binding.

The Relative Joint Exposure After iv and IA Drug Administration

In this *in vivo* experiment, relatively large doses were administered iv to enable determination of the $F_{iv/IA}$ of DTZ. In parallel to oral bioavailability, the $F_{iv/IA}$ was defined as the ratio between the *AUCs* of the SF profiles after iv and IA administration, respectively, and corrected for the actual doses administered. Based on the DTZ SF profiles (Fig. 3) obtained following iv and IA administration and the assumption of linear kinetics, an average $F_{iv/IA}$ of 0.04 % (±0.01) (n=4) was calculated, using Eq. 1 (Residual AUC estimates <12%).

Table I. Demographic Data and Pharmacokinetic Parameters of DTZ in Synovial Fluid and Serum After IA and iv Administration of 3.9 mg/kg to Horses (n=5)

Horse no.	H1	H2	H3	H4	H5	Mean (%RSD)
Weight (kg)	475	439	331	338	543	NC
Gender	Gelding	Gelding	Gelding	Gelding	Mare	NC
IA administration of DTZ						
Serum						
C_{max} (µg/ml)	NC	10.5	4.8	7.7	5.2	NC
t_{max} (min)	NC	119	90	92	120	NC
AUC (µg×min/ml)	NC	2,497	1,292	1,636	1,579	1,559 (28)
$t_{½,\ apparent}$ (min)	NC	136	91	136	111	119 (18)
SF						
AUC × 10^{-6}(µg×min/ml)	NC	5,208	4,767	4,428	5,933	5,078 (13)
$F_{iv/IA}$ (%)	NC	0.05	0.02	0.04	0.03	0.04 (37)
V_{joint} (ml)	NC	22	18	18	23	20 (13)
$t_{½,\ joint}$ (min)	NC	42	40	50	39	43 (12)
iv administration of DTZ						
Serum						
AUC (µg×min/ml)	1,661	2,555	2,135	2,066	1,483	1,938 (20)
$V_{central}$ (l)	NC	34	39	34	44	38 (13)
$CL_{central}$ (ml/min/kg)	NC	1.5	2.2	2.0	2.2	2.0 (16)
$t_{½,\ central}$ (min)	97	100	93	97	101	98 (3)
SF						
C_{max} (µg/ml)	10.6	12.3	4.7	11.8	14.0	NC
t_{max} (min)	61	29	30	29	21	NC
AUC (µg×min/ml)	1,869	2,384	822	1,687	1,713	1,609 (33)
$t_{½,\ apparent}$ (min)	107	109	79	90	78	93 (16)

NC Not Calculated

DISCUSSION

Based on the experience gained in the present study, it appears that the horse is an appropriate animal model for this type of pharmacokinetic study. It allows for easy access to the radiocarpal joint, and frequent sampling of relatively large serum volumes (10 ml) is possible. Further, moderate sample volumes (0.4 ml) can be frequently withdrawn from the synovial compartment. The horse is conscious during the entire experiment and reasonably co-operative. Housing requirements and the need for involvement of a veterinarian might be considered as disadvantages.

Comparable serum half-lives ($t_{½,central}$ and $t_{½,apparent}$) were obtained following iv and IA injection of DTZ (Table I). For comparison, the DTZ plasma half-lives in humans are in the same range (1–2 h, (21)). The apparent half-life found in SF for DTZ after iv administration is of the same order of magnitude. A similar *in vivo* behavior has been reported for tenoxicam in humans (22).

The data in Table I reveal that the model drug is apparently cleared slightly faster from the synovial compartment after IA injection than after iv injection (43 min *versus* 93 min). The latter observation may most likely be ascribed to the large concentration gradient between this rather small joint compartment and the central compartment after the IA administration. However, it can not be excluded that in the case that sampling had been performed over a longer period of time, a linear IA SF profile parallel to the iv SF profile could have emerged. Only limited studies reporting SF half-lives in the horse have been found. Experiments using the drug Ceftiofur have revealed a SF half-life of 5.1 hr upon IA administration (18). Compared to the longer SF half-life of Ceftiofur, this is in accordance with the fact that the plasma half-life of ceftiofur (6.7 h, (18)) exceeds that of DTZ (98 min). SF concentration data of carpofen and ketoprofen have been reported (23). However, the number of data points did not allow for determination of SF half-lives. Reported NSAID SF half-lives in humans exceed that of DTZ calculated in the present study, partly reflecting the longer plasma half-lives of these highly protein-bound NSAIDs (24,25).

Time to reach maximum DTZ concentration, t_{max}, in the synovial fluid after iv administration is short (21–61 min),

Table II. Micro-constants Obtained From Pharmacokinetic Analysis of DTZ Concentrations in Serum and Synovial Fluid After Dosing 3.9 mg/kg iv and IA to Horses (n=4). RSDs (%) Given in Brackets

Micro-constants	H2	H3	H4	H5	Median
k_e (min^{-1})	0.0194 (15)	0.0183 (20)	0.0203 (20)	0.0273 (25)	0.0199
k_{jc} (min^{-1})	0.0156 (3)	0.0161 (4)	0.0180 (4)	0.0172 (5)	0.0167
$k_{cj} \times 10^6$ (min^{-1})	10 (18)	3 (24)	10 (24)	12 (31)	10
k_{pc} (min^{-1})	0.0199 (28)	0.0228 (46)	0.0204 (34)	0.0193 (31)	0.0202
k_{cp} (min^{-1})	0.0206 (47)	0.0143 (86)	0.0199 (62)	0.0389 (54)	0.0203

indicating rapid distribution of this small-molecule drug between serum and SF. Rapid distribution into SF was also reported for another small-molecule drug, naproxen, after oral administration to humans (26).

A system of differential equations has been used for describing the transport of DTZ between the joint and the central compartment. These equations define that the disappearance rate of DTZ from the joint compartment and the appearance rate of DTZ in the central compartment are identical. Fig. 4 shows the three-compartment lag-time model predicted and the observed profile for a representative horse (H2). Here it is clearly demonstrated that the differential equations applied can be used for describing the data obtained and further indicate that the rate of drug appearance of DTZ in the systemic circulation equals the rate of disappearance from the joint compartment. Importantly, this observation implies that aspects of the IA fate of drug substances in addition to DTZ can be determined from serum data in cases where only sparse SF sampling or no SF sampling are possible. However, the general validity of the pharmacokinetic model has to be established through determination of the *in vivo* fate of several drugs with different physiochemical properties as well as protein affinities.

A mean $F_{iv/IA}$ of 0.04 % (Table I, $n=4$) was calculated for DTZ. Previously, a quite similar relative synovial availability of 0.06% of ceftiofur following IA and iv injection of different doses of this antibiotic in the horse has been estimated (18). At steady state, the fraction of an iv drug dose that reaches a particular joint, f_{joint}, can be estimated from Eq. 8 (5):

$$f_{joint} = \frac{V_{joint} \times K_{joint}}{V_D} \quad (8)$$

where V_{joint} and V_D refer to the apparent distribution volumes of the joint and the central compartment, respectively. K_{joint} represents the joint tissue-to-plasma ratio of drug concentration, which is expected to be close to unity for major weight-bearing joints (5). Therefore, applying Eq. 8 and the volumes of distribution given in Table I estimated f_{joint} values of 0.05–0.06 % ($n=4$) are obtained. The fraction of DTZ available is close to that maximally achievable for small molecules with low degree of protein binding, since this highly water-soluble drug substance exhibits a small volume of distribution ($V_{central}$). This estimate can be used to calculate the percentage of an oral dose of other drugs that reaches a specific arthritic joint in case their oral bioavailability and volume of distribution are known. Conversely, in case the therapeutic drug concentration at the target site is known, the above considerations might be used to estimate the size of an oral dose to be given. Assuming that the $F_{iv/IA}$ of DTZ in man is of the same order of magnitude as that observed in the horse, the data of the present study strongly indicate a rationale for IA administration of future anti-osteoarthritic drug candidates in case severe systemic dose-related adverse effects are seen after oral administration.

CONCLUSION

The pharmacokinetics of the highly hydrophilic anionic drug, diatrizoate, in serum and synovial fluid after iv and IA administration have been presented. It has been verified that it is possible to estimate joint disappearance kinetics of DTZ from serum appearance kinetics. The obtained results may indicate that it might be possible to determine aspects of the intra-articular fate of drug substances from serum data in case synovial fluid sampling for various reasons is inappropriate. Furthermore, the very low relative joint exposure after iv and IA drug administration ($F_{iv/IA}$) found in this study may stimulate future research in the field of intra-articular administration and depot formulation development for anti-osteoarthritic drugs.

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REFERENCES

1. Elders MJ. The increasing impact of arthritis on public health. J Rheumatol. 2000;27:6–8.
2. Goldring MB. Update on the biology of the chondrocyte and new approaches to treating cartilage diseases. Best Pract Res Clin Rheumatol. 2006;20:1003–25.
3. Steinmeyer J, Konttinen YT. Oral treatment options for degenerative joint disease-presence and future. Adv Drug Deliv Rev. 2006;58:168–211.
4. Gerwin N, Hops C, Lucke A. Intraarticular drug delivery in osteoarthritis. Adv Drug Deliv Rev. 2006;58:226–42.
5. Larsen C, Østergaard J, Larsen SW, Jensen H, Jacobsen S, Lindegaard C, *et al.* Intra-articular depot formulation principles: Role in the management of postoperative pain and arthritic disorders. J Pharm Sci. 2008;97:4622–54.
6. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: Role in arthritis. Front Biosci. 2006;11:529–43.
7. Roach HI, Aigner T, Soder S, Haag J, Welkerling H. Pathobiology of osteoarthritis: Pathomechanisms and potential therapeutic targets. Curr Drug Targets. 2007;8:271–82.
8. Burrage PS, Brinckerhoff CE. Molecular targets in osteoarthritis: Metalloproteinases and their inhibitors. Curr Drug Targets. 2007;8:293–303.
9. Wieland HA, Michaelis M, Kirschbaum BJ, Rudolphi KA. Osteoarthritis—An untreatable disease? Nat Rev Drug Discov. 2005;4:331–44.
10. Abramson S. Drug delivery in degenerative joint disease: Where we are and where to go? Adv Drug Deliv Rev. 2006;58:125–7.
11. Caldwell JR. Intra-articular corticosteroids—Guide to selection and indications for use. Drugs. 1996;52:507–14.
12. Hunter JA, Blyth TH. A risk-benefit assessment of intra-articular corticosteroids in rheumatic disorders. Drug Saf. 1999;21:353–65.
13. Holland TA, Mikos AG. Advances in drug delivery for articular cartilage. J Control Release. 2003;86:1–14.
14. Wind WM, Smolinski RJ. Reliability of common knee injection sites with low-volume injections. J Arthroplasty. 2004;19:858–61.
15. Reynolds JEF. Martindale: the extra pharmacopoeia. In Reynolds JEF, editor. London: The Pharmaceutical; 1989, pp. 863–865.
16. Chellquist EM, Nelson WO, Storflor HL. Assay and purity analysis of diatrizoate sodium in drug product by LC. J Pharm Biomed Anal. 1997;16:39–45.
17. The Danish Medicines Agency. Urografin, injektionsvæske, opløsning 30%. http://www.produktresume.dk. (accesed 07/06/09).
18. Mills ML, Rush BR, St Jean G, Gaughan EM, Mosier D, Gibson E, *et al.* Determination of synovial fluid and serum concentrations, and morphologic effects of intraarticular ceftiofur sodium in horses. Vet Surg. 2000;29:398–406.
19. Lerner HH. Diatrizoic acid. In: Florey K, editor. Analytical profiles of drug substances. New York: Academic; 1975. p. 137–67.

20. Yamaoka K, Nakagawa T, Uno T. Application of Akaikes Information Criterion (Aic) in evaluation of linear pharmacokinetic equations. J Pharmacokinet Biopharm. 1978;6:165–75.
21. Feldman S, Hayman LA, Hulse M. Pharmacokinetics of low-dose and high-dose intravenous diatrizoate contrast-media administration. Invest Radiol. 1984;19:54–7.
22. Bird HA, Allen JG, Dixon JS, Wright V. A pharmacokinetic comparison of tenoxicam in plasma and synovial-fluid. Br J Rheumatol. 1985;24:351–6.
23. Armstrong S, Tricklebank P, Lake A, Frean S, Lees P. Pharmacokinetics of carprofen enantiomers in equine plasma and synovial fluid—a comparison with ketoprofen. J Vet Pharmacol Ther. 1999;22:196–201.
24. Day RO, McLachlan AJ, Graham GG, Williams KM. Pharmacokinetics of nonsteroidal anti-inflammatory drugs in synovial fluid. Clin Pharmacokinet. 1999;36:191–210.
25. Owen SG, Francis HW, Roberts MS. Disappearance Kinetics of Solutes from Synovial-Fluid After Intraarticular Injection. Br J Clin Pharmacol. 1994;38:349–55.
26. Bruno R, Iliadis A, Jullien I, Guego M, Pinhas H, Cunci S, *et al.* Naproxen kinetics in synovial-fluid of patients with osteoarthritis. Br J Clin Pharmacol. 1988;26:41–4.