

PHARMACOKINETICS OF DICLOFENAC IN SHEEP FOLLOWING INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION

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دواء دايلوفيناك هو أحد الأدوية اللاستيرويدية المضادة للإلتهاب الشائعة الاستخدام لعلاج كل من البشر والحيوانات. تهدف هذه الدراسة إلى التعرف على حركة دواء دايلوفيناك في الخراف بعد جرعة وريدية أو عضلية، حيث تم تعاطي الدواء (1مغ/كغ) لعشرة من الخراف التجديدية السليمة وريدياً أو عضلياً (عدد 5 لكل منها). وتم جمع عينات دم (5ممل) وفصل مصصل الدم وتحليله بواسطة كروماتوغرافيا السوائل عالية الكفاءة مع كشف فوق بنفسجي. وتم حساب مؤشرات حركة الدواء بالتحليل اللاجراتي. وقد تبين أن دواء دايلوفيناك قد تم إخراجها بسرعة من الخراف بعمر نصف حيوي يتراوح بين 2-3 ساعات لكل من طريقتي التعاطي. وكانت التصفية الكاملة لدواء دايلوفيناك بعد الحقن الوريدي والعضلي 87.86 ± 10.78 و 85.69 ± 18.23 مل/ساعة/كغ على التوالي. ومن الواضح أن الإتاحة الحيوية المطلقة لدواء دايلوفيناك عضلياً كانت ممتازة ($\sim 100\%$)، مما يدل أن الجرعة المماثلة يمكن إعطاؤها عضلياً بدلاً من إعطائها وريدياً. ومن النتائج تبين أنه يجب إعطاء الدواء 2-3 مرات يومياً للخراف للحصول على تراكيز علاجية. ولا تزال هناك حاجة إلى إجراء المزيد من الدراسات لتقييم طرق التخلص من الدواء في الخراف بما في ذلك تكون مستقلبات الدواء وأهمية التدوير الكبدى المعوى في هذه العملية.

Diclofenac is an important nonsteroidal anti-inflammatory drug that is commonly used in both humans and animals. The aim of this work was to examine the pharmacokinetics of diclofenac in sheep after intravenous and intramuscular dosing. Diclofenac (1 mg/kg) was administered to ten clinically healthy-male Najdi sheep intravenously or intramuscularly (n = 5 each). Blood samples (5 ml) were collected and serum was separated for drug analysis by high performance liquid chromatography with UV detection. Diclofenac pharmacokinetic parameters were determined by non-compartmental analysis. Diclofenac is quickly eliminated from sheep with a terminal $T_{1/2\lambda}$ of 2-3 hr for both routes of administration. Total diclofenac clearance after intravenous and intramuscular administration was 87.86 ± 10.78 and 85.69 ± 18.23 ml/hr/kg, respectively. Apparently, the absolute bioavailability of IM diclofenac is excellent ($\sim 100\%$), which indicates that a similar dose could be given IM instead of IV if needed. Given this data, diclofenac should be administered 2-3 times daily in sheep (via these routes) to maintain therapeutic concentrations. Additional studies are needed to evaluate the route of elimination of diclofenac in sheep including metabolites formation and the significance of enterohepatic circulation in this process.

Key words: Diclofenac, pharmacokinetics, sheep.

Introduction

The nonsteroidal anti-inflammatory drugs (NSAIDs) are a chemically diverse group of agents that share similar pharmacologic properties and are

widely used to control pain and inflammation. These agents exert their effects through inhibiting cyclooxygenases (COX) 1 and 2 to varying degrees (1, 2). Diclofenac (Diclo) (2-[(2,6-dichlorophenyl) amino] phenylacetate) is a commonly employed NSAID in human and veterinary practices (3). It is used for the management of post-traumatic pain, postoperative wound hyperalgesia, pain associated with movement and swelling and for the relief of joint lameness in horses in veterinary medicine (4, 5). Diclofenac is very effective in the treatment of acute aseptic arthritis and myositis in cattle and

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buffaloes (6, 7), and is also an effective agent in reducing the signs of pain (reduces the time spent trembling or in abnormal postures) suffered by lambs at castration (8, 9). Furthermore, combinations of diclofenac sodium with fluconazole may prove to be useful as chemotherapeutic agents for the treatment of *Candida Albicans* infections (10). It is very effective against *Brucella* species when combined with streptomycin, rifampicin or tetracycline in vitro and could be a promising drug to protect against schistosomiasis which is very common in sheep (11, 12).

Some work has previously been carried out examining the pharmacokinetics of Diclofenac in animals including dogs, rats, pigs, and camels (13-17). Literature review has revealed a lack of any report about diclofenac pharmacokinetics in sheep. Therefore, the objective of this study was to describe the pharmacokinetic behavior of diclofenac in sheep following single intravenous and intramuscular administration.

Materials and Methods

Reagents and Chemicals:

Diclofenac sodium and flufenamic acid were purchased from Sigma-Aldrich chemical company (St. Louis, MO, USA). HPLC grade methanol, acetonitrile, diethyl ether, and anhydrous sodium acetate were obtained from BDH Chemicals Ltd. (Poole, UK). All other reagents and chemicals used in the assay were of the highest purity available for analytical research; water was a Milli-Q quality.

Experimental Design:

All animals were maintained in accordance with the recommendation of the "Guide for the Care and Use of Laboratory Animals" approved by King Faisal University Animal Care and Use Committee. Ten clinically healthy-male Najdi sheep, weighing 49.3 ± 2 Kg and aged 2-3 years were housed during the study in individual pens at an ambient temperature ranging between 35 and 38 °C, Hay and water were provided *ad libitum*. After three weeks of acclimatization, they were divided into two groups of 5 animals each. Diclofenac (Voltaren[®], Novartis) 1mg/kg, was given intravenously (group A) and intramuscularly (group B). Blood (5 ml) was collected from the jugular vein through an indwelling catheter into vacutainer tubes (anticoagulant free) before treatment and at 5, 10,

15, 30, 45, 60 and 90 min and at 2, 2.5, 3, 4, 5, 6, 7, 9, 12, and 24 hr after dosing. Serum was separated by centrifugation of blood at 2500 g for 10 min and stored at -20 °C until analysis.

Diclofenac Assay:

Determination of diclofenac in serum was done by high performance liquid chromatography system (HPLC) as it has previously been described with some modifications (18). Briefly, diclofenac was extracted from serum as follows: to 1 ml of serum sample in a 15 ml capped glass tube, 100 µl of the internal standard solution (5 µg/ml flufenamic acid prepared in deionized water), and 250 µl of 0.15 M phosphoric acid were added. The samples were then vortexed for 30 seconds, then extracted with 8 ml of diethyl ether. After centrifugation at 3000 g for 10 min, the supernatant organic layer was transferred into glass centrifuge tubes and evaporated to dryness under gentle stream of nitrogen. The residue was dissolved in 150 µl of the mobile phase and 100 µl injected onto the column. A calibration curve was prepared with each batch of test samples by spiking known amounts of diclofenac to blank sheep serum and treating in the same manner described above. HPLC system consisted of a Waters model 717 autosampler, model M-600 HPLC pump, and model M-2487 dual UV absorbance detector set at $\lambda=280$ nm. The mobile phase consisted of a mixture of 0.004 M sodium acetate-acetonitrile-glacial acetic acid (46:51:3 v/v/v) and was delivered at a flow rate of 1.5 ml/min through a stainless steel Novapak C₁₈ 4µm reverse phase column (150×4.6mm). Signal output was captured using Millennium³² software, version 3.05 (Waters Corp., Milford, MA, USA).

Pharmacokinetic Analysis:

Diclofenac pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin (version 4.1, Pharsight Corporation, Palo Alto, CA, USA). The maximum observed serum concentration (C_{max}) and the time to reach the maximum serum concentration (T_{max}) following IM administration were determined directly from the observed data. The diclofenac apparent elimination rate constant (λ_z) was estimated by linear regression analysis of the terminal portion of the log concentration-time data. Diclofenac apparent elimination half-life ($T_{1/2\lambda}$) was calculated as $\ln 2/\lambda$. The area under the concentration versus time curve (AUC) was calculated by the trapezoidal rule with

extrapolation to infinity. AUC from time 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of AUC_t and C_{last}/λ_z , where C_{last} was the last non-zero concentration. Apparent diclofenac volumes of distribution (V_z) in sheep after IV and IM administration was calculated as CL/λ_z ; whereas, the apparent volume of distribution at steady state (V_{ss}) was calculated as $(AUMC_{0-\infty}/AUC_{0-\infty}) \cdot CL$ following IV administration. To calculate V_{ss} for IM diclofenac, the mean disposition time (MDT) was first computed as being the difference between the mean resident time (MRT) and the mean input time (MIT); MIT was estimated as $1/K_a$ for a first order input, and K_a was computed as $(MRT_{IM} - MRT_{IV})^{-1}$. Given MDT, V_{ss} was then calculated as $CL/F \cdot MDT$. The apparent clearance (CL/F) of diclofenac following intramuscular administration was computed as $dose/AUC_{0-\infty}$. The absolute bioavailability of diclofenac after IM administration was estimated as $AUC_{0-\infty IM} / AUC_{0-\infty IV}$.

Statistical Analysis:

Data are presented as the mean \pm S.E. Differences in pharmacokinetic parameters of diclofenac between the two groups were assessed by an unpaired t-test on log-transformed data. Statistical significance will be assumed when $p \leq 0.05$. All calculations were performed using GraphPad Prism version 3.00 for Windows (San Diego, CA, USA).

Results

The lower limit of quantification (LLOQ) was 10 ng/ml; within-assay and between assay replicate studies gave coefficients of variation of <8%. Figure 1 presents serum concentration-time curve of diclofenac in sheep following IV and IM administration. Pharmacokinetic parameters of diclofenac are shown in table 1. The estimated first order absorption rate constant (K_a) following IM administration was $1.3 \pm 0.7 \text{ hr}^{-1}$. The calculated absolute bioavailability of diclofenac given intramuscularly in sheep is $98.5 \pm 27.9 \%$, and the terminal half-life ($T_{1/2\lambda}$) after IV and IM administration is $2.84 \pm 0.87 \text{ hr}$ and $2.12 \pm 0.72 \text{ hr}$, respectively ($p > 0.05$).

Discussion

To best of our knowledge, this is the first work designed to evaluate the disposition of diclofenac in

sheep after intravenous and intramuscular administration. Our study has shown that diclofenac is quickly eliminated from sheep with a terminal $T_{1/2\lambda}$ of 2-3 hr after IV and IM administration. Given this, the drug should be administered 2-3 times per day in sheep. The slight difference in the elimination rate constant (λ_z) between the two routes (0.34 hr^{-1} with IV vs. 0.43 hr^{-1} with IM) was not statistically different ($p > 0.05$), although it might be due to an enterohepatic circulation of diclofenac with IV administration resulting in a less steep terminal slope. With IV injection, more drug may get into the liver and more can be secreted through bile and get enterohepatically reabsorbed; while with IM injection drug absorption is slower and at a time smaller amount may reach the liver and less secreted into the bile. This however needs to be specifically further evaluated.

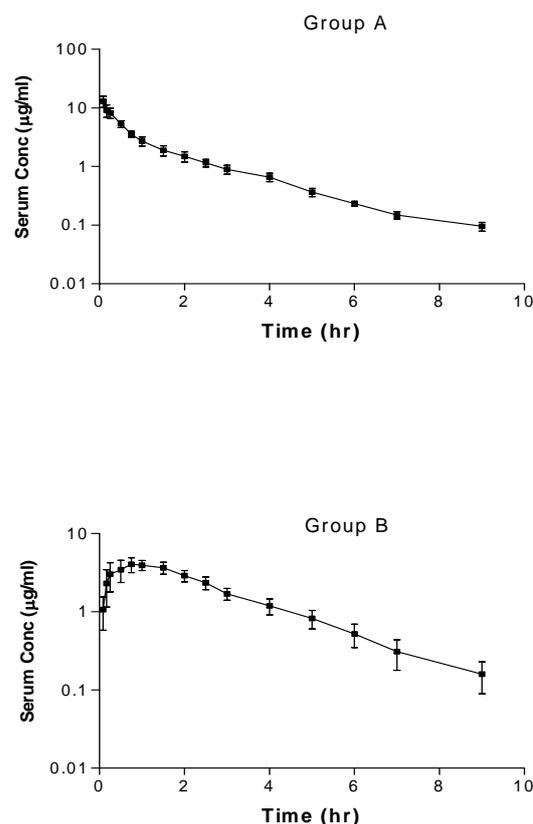


Figure 1. Diclofenac serum concentration in healthy Sheep following intravenous (upper panel, n=5) and intramuscular (lower panel, n=5) administration. The data is presented as mean \pm SE.

The difference in terminal $T_{1/2\lambda}$ may have also contributed to a larger calculated V_z value following IV injection. Based on the equation, it is possible if λ_z is different between the two administration routes: $V_z = F \cdot \text{Dose} / \text{AUC} \cdot \lambda_z$. Therefore, V_{ss} is a more reliable estimate of drug's volume of distribution since it is independent of the rate elimination constant (Table 1). Apparently, the absolute bioavailability of IM diclofenac is excellent (~100%), which indicates that a similar dose could be given IM instead of IV if needed.

Marked differences in the pharmacokinetic properties of diclofenac exist between various species because of the enterohepatic circulation. For example, diclofenac is extensively metabolized in

humans, monkeys, and camels, but not in dogs (16, 19, 20). The drug is also subject to enterohepatic recirculation in dogs but not in man (19). Table 2 shows a comparison of some diclofenac pharmacokinetic parameters in human, sheep, camel, and minipig. Similar to camel, diclofenac appears to have smaller volume of distribution in sheep, which may be due to its low partition into non-aqueous compartments (21) and its possible high serum proteins binding. Additional studies however are needed to assess the route of elimination of diclofenac in sheep including metabolites formation and the significance of enterohepatic circulation; evaluating diclofenac serum/plasma protein binding in sheep is also needed.

Table 1. Pharmacokinetic Parameters of Diclofenac in Sheep*

| <i>Parameter</i> | Treatment | |
|---------------------------------|------------------|----------------|
| | IV | IM |
| T_{\max} (hr) | 0.00 ± 0.00 | 0.90 ± 0.20 |
| C_{\max} (µg/ml)** | 15.30 ± 3.30 | 4.80 ± 0.88 |
| λ_z (hr ⁻¹) | 0.34 ± 0.08 | 0.43 ± 0.08 |
| V_z/F (ml/Kg) | 390.80 ± 154.70 | 226.58 ± 51.25 |
| V_{ss}/F (ml/kg) | 200.46 ± 55.42 | 232.58 ± 43.52 |
| CL/F (ml/hr/kg) | 87.86 ± 10.78 | 85.69 ± 18.23 |
| $AUC_{0-\infty}$ (µg.hr/ml) | 12.24 ± 1.81 | 13.88 ± 2.66 |

*For IV administration, $F=1$. T_{\max} , time to maximum serum concentration; C_{\max} , maximum serum concentration; λ_z , apparent terminal elimination rate constant; V_z/F , volume of distribution z ; V_{ss}/F , volume of distribution at steady state; CL/F , apparent drug clearance; $AUC_{0-\infty}$, area under the serum concentration-time curve. Data are presented as mean ± S.E.

** $p < 0.01$.

Table 2. Diclofenac Pharmacokinetic Parameters in Different Species after IV Administration*

| <i>Parameter</i> | Species | | | |
|-----------------------------|----------------|--------------|--------------|----------------|
| | Human | Sheep | Camel | Minipig |
| V_{ss} (ml/kg) | - | 200.5 | 320 | - |
| CL (ml/hr/kg) | 252.0 | 87.9 | 170 | 57.0 |
| $AUC_{0-\infty}$ (µg.hr/ml) | 3.3 | 12.2 | 14.5 | 35.5 |

*Dose = 50 mg in human and Minipig, 1 mg/kg in sheep, and 2.5 mg/kg in camel. References: (15, 16, 22).

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