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Preliminary Study on the Effect of Flunixin Administration on Pharmacokinetics of Cefquinome in Diseased Cattle Calves

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Abstract

Cefquinome is one of the fourth generations of cephalosporins developed for veterinary use in treatment of respiratory diseases that are considered the second causes of death in calves. Non-steroidal anti-inflammatory drugs such as flunixin (NSAIDs) are widely prescribed with antibacterial agents in multiple drug prescriptions. The present study aimed to investigate the effect of co-administration of flunixin on the disposition kinetics of cefquinome after intramuscular injection in 10 diseased calves (*Pasturella heamolytica* infected). Cefquinome was injected in a single dose (2 mg/kg BW) in 5 diseased calves alone and coupled with flunixin (1mg/kg BW) in the other 5 diseased calves. Blood samples (5 mL) were collected from the right jugular vein of each calve immediately before treatment and at intervals of 0, 5, 10, 15 and 30 min, 1, 2, 4, 6, 8, 12, and 24 hours, 48, 72, 96 and 120 h (5 days) after cefquinome administration. The obtained samples were assayed with the plate microbiological assay method using *Sarcina lutea* (ATCC 9341) as test organism. The plasma cefquinome concentration at 5 min after intramuscular injection of cefquinome alone and coupled with flunixin was 0.27 ± 0.05 µg/mL and 0.35 ± 0.12 µg/mL, respectively and reached the highest concentration (1.02 ± 0.12 µg/mL and 1.02 ± 0.08 µg/mL) at 1 h, respectively. The obtained data showed no significant effect of coupled administration of flunixin with cefquinome on either concentration or peak concentration of cefquinome in plasma of diseased calves. It is concluded that flunixin can be used successfully with cefquinome in treatment of bacterial respiratory diseases associated with inflammation in calves.

Keywords: Flunixin, Cefquinome, Pharmacokinetics, Diseased Calves, Plat Microbiological Assay

Introduction

Cefquinome is an aminothiazol cephalosporin which has been commonly used for treatment of respiratory diseases, calf septicemia and foot rot in cattle [1].

Pharmacokinetic studies of cefquinome have been conducted in lactating goats with and without experimentally induced *Staphylococcus aureus* mastitis and with tolfenamic acid in sheep [2,3]. In addition, pharmacokinetic/ pharmacodynamic (PK/PD) dose optimization of cefquinome in cattle [4], buffalo calves [5], goats [6], piglets [7], sheep [1,8], rabbits [9], horses [10], camels [11] and pigs [12] have also been reported.

Respiratory diseases are the second causes of death and losses after scours in un-weaned heifer calves. In the last 20 years, respiratory problems resulted in nearly 21% of all newborn calf losses [13]. Flunixin is a non-steroid anti-inflammatory drug (NSAID) used for analgesic and antipyretic purposes in a variety of mammalian species. NSAIDs inhibit cyclo-oxygenase (COX1), which is responsible for the synthesis of prostaglandins (PGs) from arachidonic acid [14]. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed with antibacterial agents in multiple drug prescriptions [15]. They are frequently recommended as synergistic therapy with antibacterial for treating various bacterial infections accompanied by inflammatory conditions in animals.

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Pharmacokinetic values are usually obtained in healthy animals, whereas drugs are frequently administered to diseased animals. However, there are no enough studies concerning the effect of flunixin administration on pharmacokinetics of cefquinome in diseased cattle calves. The present study was conducted as a preliminary investigation of the effect of flunixin administration on the kinetics of cefquinome after IM injection in calves infected with *Pasturella*.

Material and methods

Animals

The protocol was approved by the Animal Care and Use Committee of Cairo University. Calves with respiratory signs were diagnosed by veterinarian based on the clinical signs which included: difficult breathing, nasal discharge, fever over 40°C, diminished or no appetite (off-feed) [16]. Bacteriological isolation and identification has been done with nasal and tracheal swaps [17]. Blood smears from affected animals were stained with methylene blue stains. The organisms appeared as Gram-negative, bipolar-staining short bacilli and the biochemical identification of the bacterial isolates was conducted according to MacFadinn's method [18]. For reliable identification and comparison of results, the AIPE 20 system (Biomariux France) was used. *P. haemolytica* is able to produce a narrow zone of haemolysis on Blood agar and grow on McConkey agar, but cannot produce indole, while *Pasteurella multocida* is unable to produce haemolysis on Blood agar and cannot grow on MacConkey, but able to produce indole. Ten diseased calves with respiratory signs (3-6 months age) with BW ranged between 40-70 kg were obtained from a local private farm at El-Tal Elkebeer. Calves were housed together at Cairo University in one large indoor stall and fed Berseeme clover (*Trifolium alexandrinum*) which also is known as Egyptian clover and concentrates with free access to food and water.

Experimental design

Five diseased calves received a single IM injection of cefquinome (2 mg/kg BW) that was injected into the left neck area, while the other 5 diseased calves received a single IM injection of cefquinome sulfate 2 mg/kg BW with flunixin 1 mg/kg BW.

Calves had free access to water, fresh hay and concentrates for one hour following IM injection during the whole study period. Blood samples (5 mL) were collected from the right jugular vein of each calf into clean sterile heparinized centrifuge tubes (6 mL) immediately before treatment and at intervals of 5, 10, 15 and 30 min, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h (5 days) after cefquinome sulfate administration. The collected samples were centrifuged at 3000×g for 15 min, and the plasma was harvested and stored at −70°C until analyzed within 3 months for cefquinome concentration determination. Cefquinome concentrations are stable for at least 90 days at −70°C and for three freeze-thaw cycles [2,18].

Microbiological assay (MA)

Cefquinome was assayed in plasma using qualitatively standard microbiological assay method [19]. There was no significant difference in the efficiency of Microbiological and high performance liquid chromatography (HPLC) assay method in determination of cefquinome plasma concentration [2,16], therefore in the present work we used the microbiological assay method which is more available and of low cost. The protein binding percentage was determined using the following equation [20]:

$$\text{Binding \%} = \frac{\text{Zone of inhibition in buffer} - \text{Zone of inhibition in plasma}}{\text{Zone of inhibition in buffer}} \times 100$$

Statistical analysis

Statistical analysis was performed using SPSS (SPSS version 21.0 for Windows, IBM Corp., Chicago, IL, USA). Student *t*-test was used to compare means of diseased and flunixin co-administration on blood concentration and kinetic parameters. Data were expressed as mean ± SD and results with $P \leq 0.05$ were considered significantly different.

Results

Standard curves

The strain of *Sarcina lutea* (ATCC 9341) was found to be an appropriate test microorganism because of its sensitivity to cefquinome and its capacity to form sharply defined inhibition zone allowing accurate measurements. The lower limit of quantification of the assay in plasma was 0.07 µg/mL. Negative control samples did not produce bacteria inhibition.

Intramuscular injection of cefquinome

During the experimental period there was no adverse effect or toxic manifestations post intramuscular (IM) administration of cefquinome (2 mg/kg BW) alone or coupled administered with flunixin (1 mg/kg BW) in diseased calves. The mean plasma cefquinome concentration–time relationship following a single IM injection of 2 mg/kg BW alone or coupled administered with flunixin (1 mg/kg BW) in diseased calves followed compartmental model and presented as a semilogarithmic plot in Figure (1).

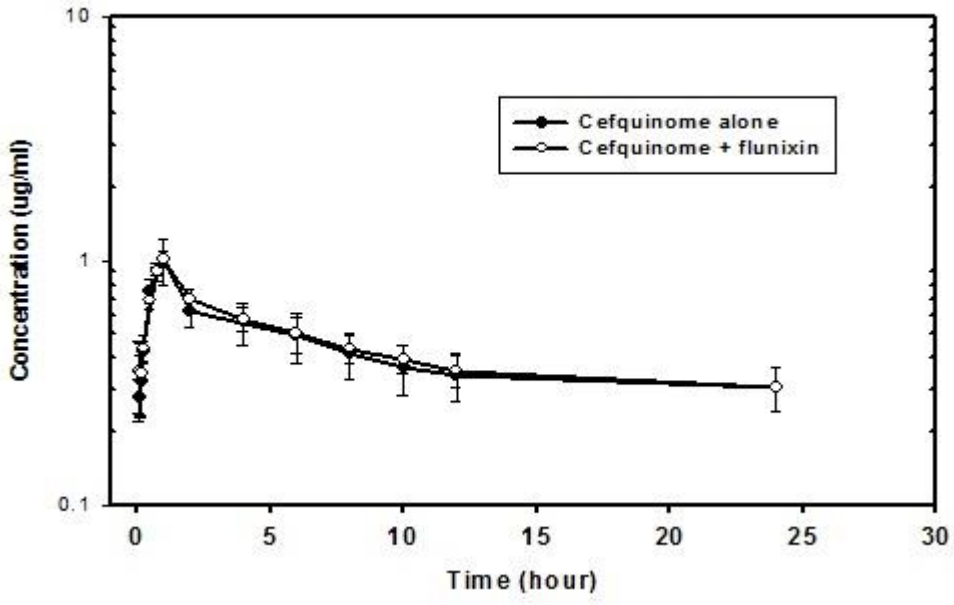

Figure 1: Semilogarithmic graph depicting the time concentration relationship after intramuscular injection of cefquinome (2 mg/kg BW) alone or with flunixin (1 mg/kg BW) in diseased claves.

The plasma cefquinome concentration following IM injection of cefquinome alone and with flunixin at 5 min was 0.27±0.05 µg/mL and 0.35±0.11 µg/mL, respectively with the peak concentration of 1.01±0.12 µg/mL and 1.01±0.07 µg/mL, at one hour, respectively. The obtained data showed no significant effect of flunixin administration with cefquinome on either concentration or peak concentrations of cefaquinome in plasma of diseased calves. Cefquinome was detected in plasma after 24 h post IM administration in either treated calves with concentrations

exceeded 0.25 µg/mL, which was reported as cequinome MIC for more than 24 h.

Pharmacokinetic analysis data of cefquinome after a single IM administration of 2 mg/kg BW or with flunixin 1 mg/kg BW are presented in Table (1). The absorption rate constant K_{ab} (1.30 ± 0.11 1/h and 0.83± 0.30), absorption half-life $t1/2_{ab}$ (0.54 ± 0.05 h and 0.96 ± 0.428), respectively and are significantly altered by coupled administration of flunixin. No significant change was observed in the major kinetic parameters by co-administration of flunixin as clearance rate constant K_{Beta} (0.26 ± 0.005 h and 0.024 ± 0.004 h) and elimination half-life ($t1/2_{Beta}$) (27.31 ± 0.97 and 29.23 ± 6.009 h.), respectively. While, AUC inf. was 21.08 ± 5.33 µg /mLh and 23.003 ± 6.019, AUMC was 820.43 ± 312.58 µg /mLh and 971.74 ± 456.76 µg /mLh) and MRT was 38.025 ± 6.96 h and 40.41 ± 8.96 h, respectively. Cefquinome showed low protein binding percent (6.67%).

Table 1: Pharmacokinetic parameters of cefquinome after a single intramuscular injection (2 mg/kg BW) alone and with flunixin (1 mg/kg BW) in diseased calves (Mean, SD)

Parameter	UNIT	Treatment	
		Cefquinome alone	Cefquinome + flunixin
[1]A	µg/ml	21.19 ± 2.9	7.53 ± 8.83*
[2]Kab	1/h	1.3 ± 0.1	0.83 ± 0.30 *
[3]A	µg/ml	0.5 ± 0.1	0.52 ± 0.05
[4]B	1/h	0.03 ± 0.01	0.02 ± 0.01
[5]K10	1/h	0.10 ± 0.02	0.07 ± 0.03
[6]t1/2ab	H	0.54 ± 0.05	0.96 ± 0.43*
[7]t1/2β	H	27.3 ± 5.0	29.24 ± 6.01
[8]Tmax	H	0.98 ± 0.13	1.05 ± 0.20
[9]Cmax	µg/ml	0.90 ± 0.10	0.92 ± 0.04
[10]AUC 0-inf	µg/ml*h	21.1 ± 5.3	23.0 ± 6.0
[11]AUMC	µg/ml*h^2	820.4 ± 312.6	971.8 ± 456.8
[12]MRT	H	3 ± 6.97	40.40 ± 8.97

***Significance compared to cefquinome alone (P ≤0.05); [1]A: The intercept of the elimination phase with the vertical axis after parentral administration; [2]Kbeta: First order elimination rate constant for disappearance of drug from central compartment (h); [3]Alfa: rate constant for drug absorption (h-1); [4]Beta: rate constant for drug elimination (h-1); [5]K10: Rate constant for central compartment distribution (h-1); [6]T1/2ab: apparent absorption half-life (h); [7]T1/2 β : the apparent terminal plasma elimination half-life (h); [8]Tmax: The time at which the drug reached the maximum concentration afterparentral administration; [9]Cmax: Maximaum serum concentration of drug in blood after parentral administration (µg/ml); [10]AUC0-inf.: Total area under the serum drug concentration versus time curve from t=0 to t=infinity after administration of a single dose (µg.ml/h); [11]AUMC: Total area under the plasma drug concentration multiplied by time versus time curve from t=0 to t=time of last taken sample after administration of a single dose (µg.ml/h); [12]MRT: Mean residence time represents the average time from time 0 to the last quantifiable time point (tlast) (h).**

Discussion

Pasteurella species are type of bacteria that commonly infect the respiratory tract of calves causing bovine respiratory disease. *Pasteurella multocida* is one of the most common bacteria isolated from calves suffering from shipping fever pneumonia. *Pasteurella* is usually a secondary bacterial invader, meaning that a virus or some other organisms firstly weakens the immune system thus allowing invasion of *Pasteurella*. The organism is found throughout the environment and within the upper respiratory tract of cattle, but it usually does not cause disease in healthy animals [21]. There was no significant effect of coupled administration of flunixin with cefquinome on either concentration or peak concentration of cefaquinome in plasma of diseased calves. Cefquinome was detected in

plasma after 24 h post intramuscular administration in either treated calves with concentrations exceeded 0.25 µg/mL, which was reported as cequinome MIC for more than 24 h [5,22].

Following IM administration of cefquinome alone or coupled with flunixin in calves suffered from respiratory signs, there was significant effect on absorption half-life (t½Kab) (27.31± 4.973 h and 29.23±6.01 h, while, no significant effect on maximum drug concentration (Cmax, 0.91±0.102 and 0.92±0.04 µg/mL) which reached at one hour was observed. Also, all other pharmacokinetic parameters were not significantly altered. A significant increase in peak plasma concentration (Cmax) of cefquinome in sheep was reported in tolfenamic acid co-administrated (4.73 ± 0.05 µg/mL) as compared to cefquinome administration alone (4.36 ± 0.10 µg/mL) [4]. Several authors have reported an increase in the Cmax of different cephalosporins following its co-administration with anti-inflammatory drugs. The present finding was different from that reported by others who detected an increase in Cmax of cefepime following coupled IM administration with ketoprofen [23,24]. In addition, a significant increase in the Cmax of ceftizoxime following paracetamol coupled IM administration in cross-bred calves was also documented [23]. The result of the present study also differed than that reported by Carbon *et al.* [25] who stated that a significant increase in Cmax of cefazolin in rabbits following intramuscular co-administration of phenylbutazone and also increased Cmax of cefotiam and ceftriaxone, following concomitant administration of diclofenac in rabbits [26]. Also, Barot [24] reported a significant increase in the Cmax of cefpirome, following co-administration of ketoprofen in goats. The results of the present study supported the findings of Patel *et al.* [27] who reported no significant difference in the Cmax of cefepime following intramuscular co-administration of ketoprofen in goats.

In the current study, the major pharmacokinetics parameters were not significantly affected following IM administration of cefquinome with flunixin in diseased calves when compared with calves administered cefquinome alone. These results were similar to that reported by Rana *et al.* [3] who studied the effect of tolfenamic acid co-administration on pharmacokinetics of cefquinome following IM administration in sheep and rabbits. The major pharmacokinetic parameters of cefmenoxime remained unaffected following concomitant diclofenac sodium administration [26], these results supported our findings. Likewise, in sheep, goats and cow calves, no significant alterations were detected the major pharmacokinetic parameters of cefepime following its IM co-administration with ketoprofen [27]. Similar results were also reported by Barot [24] who mentioned that no alteration in the major pharmacokinetics parameters of cefpirome following co-administration of ketoprofen in goats.

In contrast, a significant increase in the elimination half-life (t1/2β) of cefazolin following co-administration of phenylbutazone in rabbits was documented [25]. In crossbred calves, a significant increase in the AUC and t1/2β of ceftizoxime was reported after co-administration of paracetamol [24]. However, a significant increase in cefepime absorption half-life (t½Kab) following co-administration with ketoprofen was detected in sheep [26].

Reports of alterations in the pharmacokinetic parameters of cephalosporin when coupled with NSAIDs could be due to differences in drug properties and animal species.

Conclusion

From the current study it is concluded that intramuscular administration of flunixin (1 mg/kg BW) could be successfully coupled with cefquinome (2 mg/kg BW) for treating of bacterial infections with an inflammatory reaction in calves suffering from respiratory diseases.

Conflict of interest

None of the authors have conflict of interest.

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الملخص العربى

دراسه مبدئيه لتأثير العلاج المتزامن للفلونكسين علي المسار الحركي للسيفكينوم في عجول الأبقار المريضه

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يعتبر السيفكينوم احد مركبات الجيل الرابع لمجموعة السيفالوسبورينز التي تستخدم في الحقل البيطري لعلاج الأمراض التنفسية التي تعتبر ثاني اسباب نفوق العجول الصغيرة. توصف مضادات الالتهاب (الغير ستيرويديه) مثل الفلونكسين على نطاق واسع مع مضادات البكتيري. يهدف هذا البحث الي دراسة تاثير العلاج المتزامن للفلونكسين كمضاد للالتهابات مع السيفكينوم علي المسار الحركي للسيفاكينوم بعد اعطاء الأخير عن طريق الحقن العضلي في مجموعتين من العجول حديثة الولادة المصابة بأمراض تنفسية نتيجة العدوى بميكروب الباستيوريلاهيموليتيكا. تم اعطاء السيفكينوم بالحقن العضلي (٢ مليجرم/كيلوجرام من وزن الحيوان في المجموعة الأولي (٥عجول) وفي المجموعة الثانية (٥عجول) تم اعطاء الفلونكسين بالحقن العضلي (١ملجرام/كيلو جرام من وزن الحيوان) وتلاها اعطاء السيفكينوم بالحقن العضلي (٢ مليجرم/كيلوجرام من وزن الحيوان). تم تجميع عينات دم من المجموعتين عند اوقات متعددة عند ٥ دقائق, ١٠, ١٥, ٣٠, ١ساعه, ٢, ٤, ٦, ٨, ١٠, ١٢, ٢٤, ٤٨, ٧٢, ٩٦, ١٢٠ ساعه (٥ ايام) بعد الحقن العضلي في كل من المجموعتين. تم قياس تركيزات السيفكينوم في بلازما الدم باستخدام الطريقه الميكروبيولوجيه *Sarcina Lutea* ميكروب عيارى (ATCC 9341) .
واوضحت النتائج ان تركيز السيفكينوم في بلازما الدم بدأ في الظهور بتركيز أمكن قياسه بعد خمس دقائق 0.06 ± 0.27 (μg/mL and 0.12 ± 0.35 μg/mL) واستمر الي ٢٤ ساعة أعلي من اقل تركيز مثبط .(0.25μg/mL) وكان اعلي تركيز للسيفكينوم عند ساعة من اعطاء الدواء بالحقن العضلي في المجموعتين علي التوالي 0.12 ± 1.02 μg/mL and) (1.02 ± 0.08 μg/mLعلى التوالى. لم تظهر النتائج تغيير معنوي في تركيز السيفكينوم علي مدار العينات المختلفة في المجموعة التي اعطيت السيفكينوم منفردا او اعطاءه متزامنا مع الفونكسين ولم تسجل ايضا النتائج تغير معنوي في معايير المسار الحركي بين المجموعتين. ويستخلص من هذه الدراسة انه لا توجد اي آثار سلبية لإعطاء الفلونكسين متزامنا مع السيفكينوم لعلاج الاصابات البكتيرية التنفسية في العجول المصابه بالتهابات وعليه يمكن ان يستخدم السيفكينوم والفلونكسين معا.