Effect of amoxicillin and cefalexin on the pharmacokinetics of diclofenac sodium in healthy volunteers

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ABSTRACT

Background: **Studies investigating the effect of antibiotics on the pharmacokinetics of non-steroidal anti-inflammatory drugs are few. Such interaction could be clinically significant in conditions like diabetic nephropathy.**

Objectives: **To study the effect of amoxicillin and cefalexin on the pharmacokinetics of diclofenac sodium when taken concomitantly.**

Subjects and Methods: **Eleven healthy subjects participated in this single dose cross-over study. Each volunteer randomly joined one of the three treatment groups and received a single dose of diclofenac sodium (50mg enteric-coated tablet), diclofenac sodium + amoxicillin 500mg capsule, and diclofenac sodium + cefalexin 500mg capsule successively with one week washout interval. Blood samples were taken immediately before, at 30 minutes and at 1, 1.5, 2, 2.5, 3, 4 and 6 hours after drug administration and analyzed using high performance liquid chromatography (HPLC) system with ibuprofen as internal standard.**

Results: **Diclofenac sodium produced a maximum concentration of 1.34 µg/ml with a half life of 0.5 hour and area under plasma concentration versus time curve up to 6 hours (AUC_{0-6}) of 1.24 µg.h/ml. Although the AUC_{0-6} and maximum plasma concentration of diclofenac sodium increased by more than 20% after co-administration of amoxicillin, this increase is not statistically significant. Cefalexin, on the other hand, when given with diclofenac sodium significantly increased the AUC_{0-6} and maximum plasma concentration of diclofenac by 51.9% and 68.5% respectively.**

Conclusion: **Amoxicillin and cefalexin can change some of the pharmacokinetic parameters of diclofenac tablet when administered concomitantly and cefalexin did that to a greater extent. Such interaction must, therefore, be considered in conditions where diclofenac might be harmful.**

Keywords: diclofenac, amoxicillin, cefalexin, pharmacokinetics, interaction.

تأثير الأموكسيسلين والسفالكسين على حرائك الدايكلوفيناك صوديوم في المتطوعين الاصحاء

خلفية الدراسة: أن الدراسات التي تقصت تأثير المضادات الحيوية على حرائك الادوية المضادة للالتهاب غير الستيرويدية قليلة. ان مثل هذا التداخل قد يكون مهما" سريريا" في حالات مثل اعتلال الكلية السكري.

الهدف من الدراسة: دراسة تأثير الاموكسيسلين والسفالكسين على الحرائك الدوائية المختلفة للدايكلوفيناك صوديوم عند اعطائهما معا"

ألأشخاص وطرائق العمل: شارك أحد عشر شخصا" بالغا" في دراسة عشوائية، ثلاثية التسلسل، تعابرية وذات جرعة منفردة. وأعطي كل متطوع في أي من المجاميع الثلاثة جرعة واحدة من الدايكلوفيناك صوديوم (٥٠ ملغم قرص مغلف للأمعاء) والدايكلوفيناك صوديوم + أموكسيسلين (كبسول ٥٠٠ ملغم) والدايكلوفيناك صوديوم + سيفالكسين (كبسول ٥٠٠ ملغم) بشكل تتابعي بينهم فترات تنظيف لمدة اسبوع في الاقل. أخذت عينات من الدم قبل اعطاء الادوية مباشرة ومن ثم ٣٠ دقيقة و١و ١.٥و٢و ٢.٥و٣و٤ و٦ ساعة بعد اعطاء الدواء وتم قياس مستوى الدايكلوفيناك صوديوم باستعمال جهاز الكروماتوغرافي السائل عالي الكفاءة. واستعمل الايبوبروفين كمعيار داخلي لحساب التركيز.

النتائج: بلغ التركيز الأعلى للدايكلوفيناك صوديوم في البلازما عند اعطائه بشكل أقراص ٥٠ ملغم مغلفة للأمعاء ١.٣٤ مايكروغرام/مل وبعمر نصفي بلغ ثلاثين دقيقة ومساحة تحت المنحنى (من صفر الى ٦ ساعة) ١.٢٤مايكروغرام.ساعة\مل وعلى الرغم من أن المساحة تحت المنحنى (من صفر الى ٦ ساعة) والتركيز الاعلى يزداد بأكثر من ٢٠% بعد اعطاء كبسول الاموكسيسلين ٥٠٠ ملغم، الا أن هذه الزيادة غير معتدة احصائيا". ومن

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جانب اخر، عند اعطاء السيفالكسين مع الدايكلوفيناك صوديوم زادت المساحة تحت المنحنى والتركيز الأعلى في البلازما بشكل معتد احصائيا" وبنسبة ٥١.٩% و٦٨.٥% على التوالي.

الاستنتاج: ان الاموكسيسلين والسفالكسين يمكن أن يغيرا بعض الحرائك الدوائية لأقراص الدايكلوفيناك ٥٠ ملغم المغلفة للأمعاء عند أخذهما معا". وكان للسيفالكسين التأثير الاكبر، لذا يجب أن ينتبه الى هذا التداخل في الحالات التي يمكن للدايكلوفيناك صوديوم أن يكون مضرا".

الكلمات المفتاحية: دايكلوفيناك، أموكسيسلين، سفالكسين، حرائك الادوية، التداخل الدوائي

INTRODUCTION

Drug interactions were reported to involve 16.6% of hospital admissions in one UK study.[1] Drugs most commonly implicated in causing these admissions involved low dose aspirin, diuretics, warfarin, and non-steroidal anti-inflammatory drugs (NSAIDs) other than aspirin, and the most common reaction being gastrointestinal bleeding. Antibiotics and NSAIDs are commonly prescribed together. NSAIDs have been found to modify the pharmacokinetics of antibiotics through different mechanisms. Diclofenac was found to decrease plasma concentration of amoxicillin in human and animals. [2-4] On the other hand, diclofenac increased the concentration of cephalosporins in the rabbit.[5,6] Aspirin and ibuprofen were also found to increase cephalosporin concentrations.[7,8] Other studies found no interaction between NSAIDs and antibiotics.[9,10] The subject of antibiotics affecting the kinetics of NSAIDs is much less investigated. Azithromycin and enrofloxacin were found to increase or decrease diclofenac and piroxicam concentrations.[11-13] The present study was, therefore, conducted to investigate the effect of two commonly used oral antibiotics; amoxicillin and cefalexin, on the pharmacokinetic parameters of diclofenac sodium.

SUBJECTS AND METHODS

This study was conducted at the College of Medicine, University of Basrah during the academic year 2012/2013 and its protocol was approved by the College Ethical Committee. Eleven (out of 12) adult subjects successfully completed a randomized, three-way, cross-over study. All were considered healthy based on medical history, physical examination, and routine laboratory investigations. Their mean ± SD of body mass index (BMI) was 22.53±2.38, age 22.73±3.44 years, weight 62.18±10.38 kg and height was 165.73±8.33 cm. All subjects gave their written informed consent to participate in the study. Subjects were 5 males and 6 females, non-smokers and without a history of alcohol or drug abuse. They did not take any other concomitant medications (including herbal remedies) for one week before as well as during the study. Patients with history of drug allergy, any associated disease and repeated drug use and those with any contraindication to the use of NSAIDs had been excluded. Each volunteer randomly joined one of the three treatment groups and received a single dose of diclofenac sodium (50mg enteric-coated tablet), diclofenac sodium + amoxicillin 500mg capsule, and diclofenac sodium + cefalexin 500mg capsule successively, in a cross-over design, with one week washout interval in between. Drugs were administered with 150 ml water starting from 08.00 h after an overnight fast. Intake of light food was delayed for 3 h after drug administration. Peripheral venous blood samples were taken immediately before, at 30 minutes and at 1, 1.5, 2, 2.5, 3, 4 and 6 hours after drug administration. During each session, blood samples were collected in EDTA tubes and immediately centrifuged. Plasma was separated and frozen at −15°C until further analysis.

Determination of diclofenac sodium

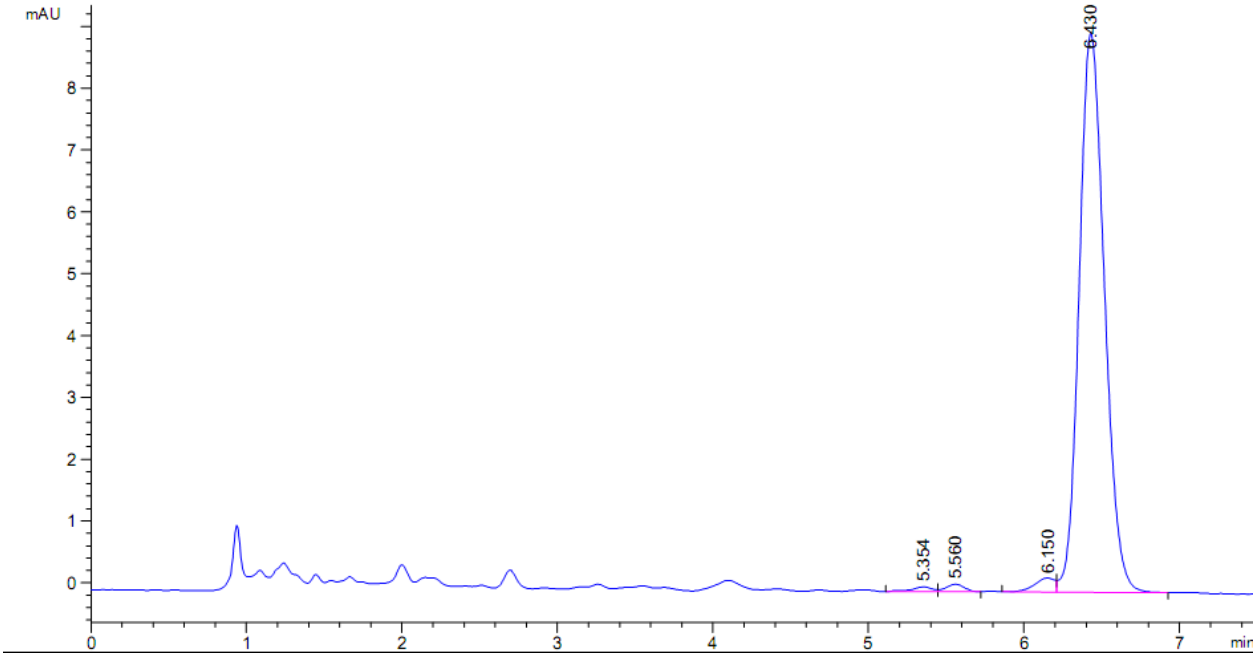
Diclofenac sodium and ibuprofen pure powders were kindly supplied by SDI, Samara, Iraq. In a 10 ml test tube, 50 µl of internal standard

solution (containing ibuprofen 2.5 µg) were added to 0.5ml of plasma. Acidification with 1 ml of 1M orthophosphoric acid was followed by extraction with 5ml of a mixture of hexane and isopropyl alcohol (90:10); then centrifugation, recovery of 3 ml of the organic layer, evaporation to dryness and dissolution of the dry residue in 100µl of the mobile phase, immediately before injection. The apparatus was an Agilent HPLC system model 1200 (USA), with variable wavelength detector. Chromatographic separation was performed using a Zorbax Eclipse XBD- C18 column. The chromatographic parameters were: pH 3.5, temperature 25°C, flow rate 1.5 ml/min, detection wavelength 276 nm. Quantitation was achieved by measurement of the peak height ratios of the drug to the internal standard. The intraday coefficient of variation (CV) of internal standard samples was 3.3% while the inter-day coefficient of variation was 8.5%. The lowest value on the calibration curve; the lower limit of quantitation, was 0.1 µg/ml.

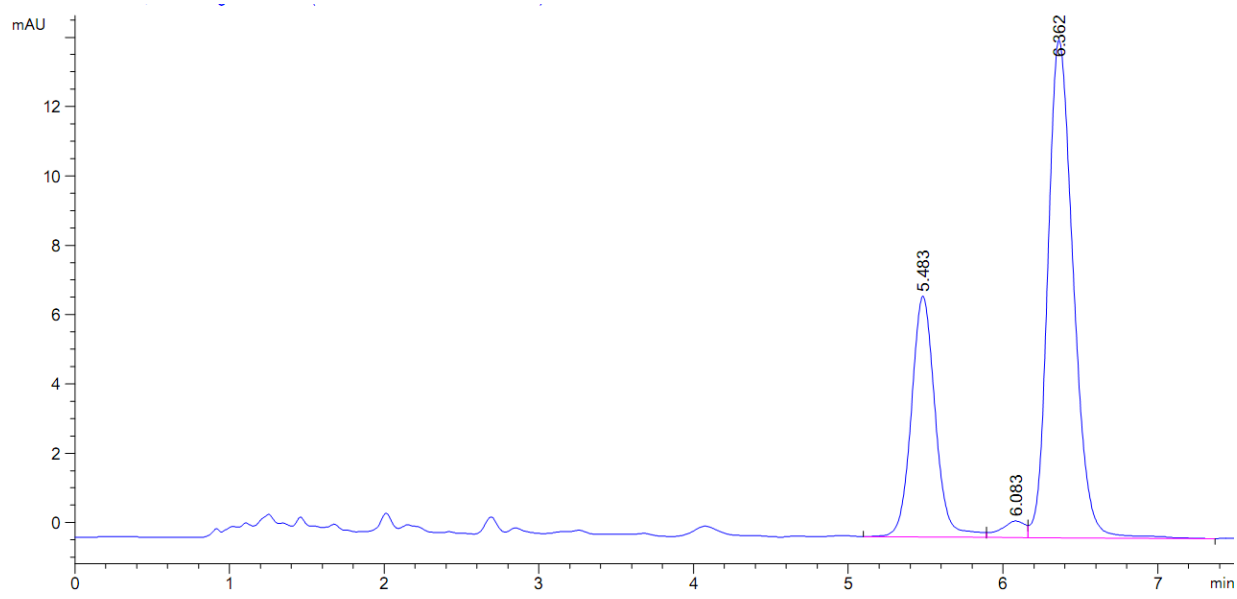
The method used for extraction of diclofenac from serum was similar to that described by Emami *et al*. [14] Differences between different parameters of each antibiotic group and that of diclofenac sodium was tested using paired t-test. A difference was considered statistically significant for p value of 0.05 and less.

RESULTS

Figure-1, shows the chromatogram of blank plasma with ibuprofen 2.5 mg/ml as internal standard **(A).** and blank plasma with diclofenac sodium at a concentration of 1 µg/ml and ibuprofen **(B).** Both compounds eluted as completely resolved peaks and no peak tailing was noticed enabling the use of peak height ratio in the calculation of the calibration curve. An optimum flow rate of 1.5 ml/min for the mobile phase resulted in the retention times of 5.3 min for diclofenac sodium and 6.3 min for ibuprofen.



(A) Ibuprofen



(B) Ibuprofen and diclofenac sodium

Fig 1. The chromatogram of blank plasma with ibuprofen at a concentration of 2.5 µg/ml as internal standard (A) and diclofenac sodium at a concentration of 1µg/ml with ibuprofen (2.5 mg/ml) (B). The retention times is 5.3 minutes for diclofenac sodium and 6.3 minutes for ibuprofen.

Diclofenac concentrations were plotted versus their respective time points to obtain a concentration-time curve from which the principal pharmacokinetic parameters were calculated. These include the area under the curve (AUC), maximal plasma concentration (C_{max}), time to achieve maximal plasma concentration, elimination half life and lag time.

Pharmacokinetic parameters of diclofenac sodium

The pharmacokinetic parameters of diclofenac sodium given alone as a single 50 mg enteric-coated tablet to 10 healthy volunteers is described in (table-1).

Table 1. Pharmacokinetic parameters of diclofenac sodium in 10 healthy volunteers

Parameters (n = 10)	Mean	Standard deviation
AUC0-6 (µg.hr/ml)	1.24	0.59
C_{max} (µg /ml)	1.34	0.67
T_{max} (hr)	2.25	1.16
$t_{1/2}$ (hr)	0.50	0.12
Lag time (hr)	1.95	1.07

Concomitant administration of amoxicillin with diclofenac sodium resulted in an increase in AUC_{0-6} and C_{max} of diclofenac sodium by 27.4% and 22.1%; and a decrease in T_{max}, $t_{1/2}$ and lag time by 24.2%, 27.8% and 24.3% respectively. However, these changes are not statistically significant except the change in half-life (Tables-2 and 4) (figure-2).

Table 2. Pharmacokinetic parameters of diclofenac sodium when given as 50 mg enteric-coated tablets alone and in combination with 500 mg amoxicillin capsule to 8 healthy volunteers

Parameters (n = 8)	Diclofenac Mean± sem	Diclofenac + Amoxicillin Mean± sem	Percent change with respect to diclofenac alone	P value
AUC0-6 (µg.hr/ml)	1.24±0.24	1.58±0.17	+27.4%	0.135
C_{max} µg /ml	1.36±0.27	1.66±0.16	+22.1%	0.229
T_{max} (hr)	2.31±0.45	1.75±0.21	-24.2%	0.285
$t_{1/2}$ (hr)	0.54±0.04	0.39±0.03	-27.8%	**0.014**
Lag time (hr)	2.06±0.41	1.56±0.24	-24.3%	0.316

Cefalexin when given in combination with diclofenac sodium increased diclofenac sodium AUC_{0-6} and C_{max} by 51.9% and 68.5% respectively. This increase is statistically significant (Table-3). The changes in diclofenac sodium T_{max}, $t_{1/2}$ and lag time are not statistically significant when given with cefalexin (tables 3 and 4), (figure-2).

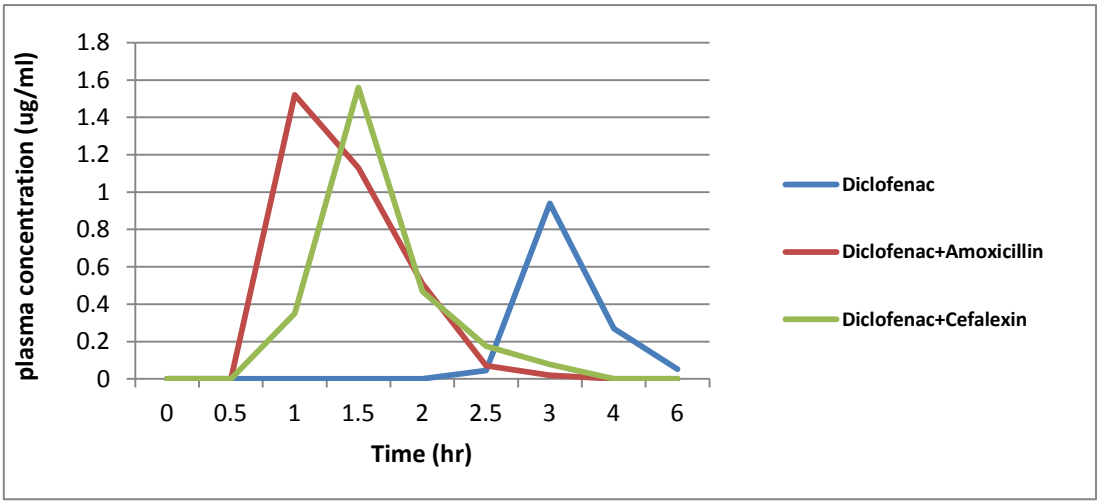
Table 3. Pharmacokinetic parameters of diclofenac sodium given as 50 mg enteric coated tablet *alone* and in combination with 500 mg cefalexin capsule to 7 healthy volunteers

Parameters N=7	Diclofenac Mean± sem	Diclofenac +cefalexin Mean± sem	Percent change with respect to diclofenac	Statistical significance
AUC_{0-6} (µg.hr/ml)	1.02± 0.19	1.55±0.17	+51.9%	**0.044**
C_{max} (µg /ml)	1.11±0.22	1.87±0.28	+68.5%	**0.028**
T_{max} (hr)	2.43±0.51	2.00±0.27	-17.7%	0.407
$t_{1/2}$ (hr)	0.52±0.05	0.47±0.05	-9.6%	0.484
Lag time (hr)	2.07±0.48	1.71±0.31	-17.4%	0.454

Table 4. Qualitative assessment of the effect of amoxicillin and cefalexin on the pharmacokinetic parameters of diclofenac sodium

Diclofenac pharmacokinetic parameters						
The type of change	**AUC_{0-6}**		**C_{max}**		**$t_{½}$**	
	+Amoxicillin	**+Cefalexin**	**+Amoxicillin**	**+Cefalexin**	**+Amoxicillin**	**+Cefalexin**
Increase	**6 +**	**6 +**	**5 +**	**6 +**	**1 +**	**2 +**
Decrease	**2 -**	**1 -**	**3 -**	**1 -**	**7 -**	**4 -**
Total	**8**	**7**	**8**	**7**	**8**	**6 + one no change**

Data are expressed as the number of volunteers showing an increase or a decrease in the pharmacokinetic parameters



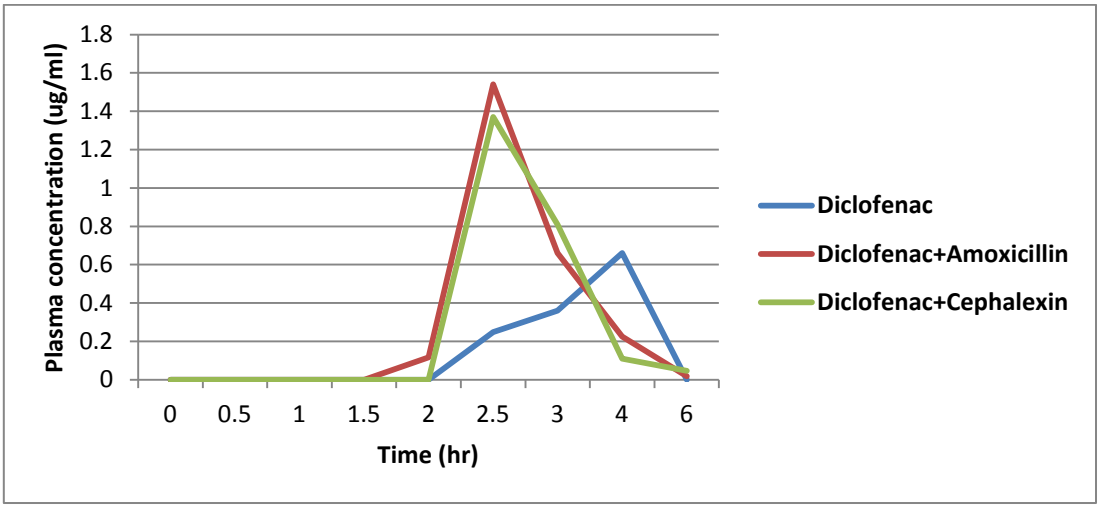


Fig 2. Examples of plasma concentration time-curve of diclofenac, diclofenac + amoxicillin, and diclofenac + cephalexin from two healthy volunteers

DISCUSSION

Several studies have investigated the interaction between NSAIDs and antibiotics. Most of them studied the extent to which NSAIDs can alter the pharmacokinetic parameters of the antibiotics. The results of these studies are variable depending on the type of antibiotics, type of NSAIDs and the species used for the study. Diclofenac, as an example, decreased the plasma concentration of amoxicillin in human and rats,[2-4] and increased that of cephalosporins and tetracycline in different animal species.[5-7],[15] Few studies investigated the effect of antibiotics on plasma concentration of NSAIDs with contradictory results. Kumar *et al* [13] found that enrofloxacin decreased the plasma concentration of diclofenac while Rahal *et al*[11] showed that the same antibiotic; enrofloxacin, increased diclofenac plasma concentration. Therefore, the present study is an attempt to study the effect of two commonly used antibiotics; amoxicillin and cefalexin, on the pharmacokinetic parameters of diclofenac sodium. Changing the therapeutic level of diclofenac might have an important impact on

the profile of its toxicity, particularly in diabetics with compromised renal function. The present study showed that both amoxicillin and cefalexin enhanced the C_{max} and AUC_{0-6} of diclofenac but cefalexin did that to a greater extent than amoxicillin. There are a number of speculations regarding the possible mechanism of this interaction. Such interaction could be at the level of the kidney where both drugs may compete for the same secretory mechanism in the renal tubules resulting in higher plasma concentration.[2] Oh and Han[15] found that renal clearance of tetracycline was reduced by diclofenac and naproxen. Although the protein and tissue binding of amoxicillin and cefalexin are not high, displacement of a highly protein bound drug with high tissue affinity like diclofenac, is a possibility.[15-17] Interaction at the level of the liver is less likely because both antibiotics are actively secreted through the kidney. However, it occurred with ceftriaxone and diclofenac in the rabbit due to increased hepatic blood flow with consequent reduced hepatic clearance.[6] It happened, sporadically, in some volunteers that diclofenac sodium could not be detected in the blood during the 6 hour period after ingestion of diclofenac sodium either alone or in combination with the antibiotics (a total of 3 volunteers). Those volunteers had been excluded from the final statistical analysis. Several explanations can be postulated. The volunteer might not have taken the diclofenac tablet; the absorption was delayed beyond the 6-hour-period, or the tablet that was taken might be defective. The first possibility was ruled out because one of these volunteers is directly related to this study. Malizia *et al* [18] found that azithromycin decreased the level of diclofenac in the first 3 hours and increased it later on. However, delayed absorption is a possibility that had not been ruled out in this study.

In conclusion, the pharmacokinetics of diclofenac could be changed by co-administration of antibiotics such as cefalexin and amoxicillin. Such interaction should be taken in consideration in conditions where diclofenac might be harmful.

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