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Vanadium *in vivo* interaction with cefatoxime

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ABSTRACT

The presence of V(IV) in penicillin and cephalosporin solutions, has been shown previously, *in vitro*, to promote the degradation of the penicillins and cephalosporins studied to their corresponding penicilloic and cephalosporanic acids. HPLC studies provided an additional evidence for the reaction mechanism. The mechanisms of V(IV) catalysis involve a ternary complex. This work was undertaken to study the consequences of this degradation, *in vivo,* upper the pharmacokinetic and pharmacodynamic of Cefotaxime, being these consequences a lower concentracion of free cefotaxime in blood, liver, spleen, kidney, lung and heart when there was intoxication with vanadium. The differences more significant between rats with vanadium and without it were observed in liver, lung and kidney.

Key words: Cefotaxime.—Vanadium.—HPLC.—Pharmacokinetic.—Pharmacodynamic.

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RESUMEN

Interacción *in vivo* del Vanadio con Cefotaxima

Anteriormente, en estudios por HPLC, hemos demostrado *in vitro,* que la presencia de V (IV) en las disoluciones de penicilinas y cefalosporinas, aumentan la degradación de éstas en sus correspondientes ácidos penicilóico y cefalosporánico. Los mecanismos de la catálisis de V (IV) involucran a un complejo ternario. En este trabajo se estudia las consecuencias de esta degradación, *in vivo,* de Cefotaxima, en sangre, hígado, bazo, riñón, pulmón y corazón, para ver las consecuencias de la intoxicación de vanadio. Las diferencias más importantes en ratas intoxicadas con vanadio se observaron en hígado, pulmón y riñón.

Palabras clave: Cefotaxima.—Vanadio.—HPLC.—Farmacocinética.—Farmacodinamia.

INTRODUCTION

The transition metal interactions to penicillins (1-5) and cephalosporins (6) were studied previously by spectrophotometric and potenciometric methods. In the case of the cefotaxime, as impurity, also for HPLC (7).

In those studies, it was observed that the effect of V(IV) on the penicillins and cephalosporins was to promote their degradation to coordination complexes of V(IV) and the corresponding penicilloic and cephalosporanic acids (see Figure 1). Other authors have studied the formation of the complex, *in vitro,* between V(IV) and penicillins and cephalosporins, by means of the reaction mechanisms, evaluating the stability constants for the interaction between V(IV) and these antibiotics.

This communication presents the way thought which the formation of the complex between V(IV) and cefotaxime affects on the concentration, distribution and stability of this cephalosporin. The importance of the formation of this complex stems from the lowest concentration of free cefotaxime in blood and organs only when takes place intoxication. This concentration could be lower than the concentration of the cephalosporin that is required to kill the microorganisms that cause the infection.

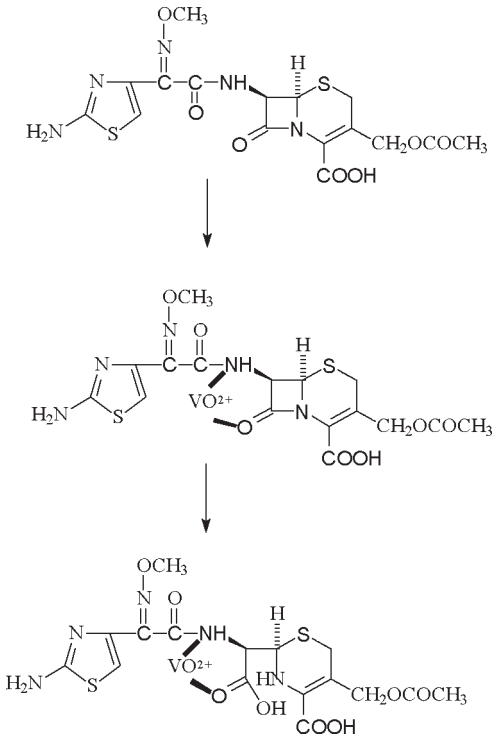

FIGURE 1. *Mechanism of hydrolytic reaction of the Cefotaxime with addition of vanadium.*

So that we could reach those results, we compared the concentration, distribution and stability of the free cefotaxime in rats without toxic with poisoned rats, after 30, 60, 90, 120, 150, 180 and 210 min. after administrating this antibiotic. This comparison was achieved by measuring the concentration of free cefotaxime in blood, liver, spleen, kidney, lung and heart homogenates of poisoned rats and rats without the toxic. The methodology applied for this purpose was HPLC since this technique facilitates the separation of the complex V(IV)-cefotaxime, the corresponding V(IV)-cephalosporanic acid chelate, free cefotaxime and their compounds resulting from the degradation of cefotaxime.

MATERIALS AND METHODS

Biology Materials

Seventy five Wistar rats, males of 250 to 300g weight, were used throughout this work and supplied by the warehouse of the Complutense University of Madrid.

Animals were maintained in one of the rats room of the warehouse. The temperature was 20º C, the relative humidity was 55% and the intensity of the light inside the room was 400 LUX using cycles of 12 hours of light/darkness.

Animals were distributed by groups in order to assay different treatments. One group of 35 rats with vanadium (group A), which was poisoned with a nasogastric sound administering to their everyday 4 mL of a solution of 4 mg/mL of a vanadium (III) acetylacetonate during 8 days. The groups left were arranged one of them with 35 rats (group B), and the other one with 5 rats (group C), both of them without metal.

Groups A and B were put under treatment with an intramuscular administration of an only dose of 200 mg of cefotaxime.

Group C was used as a control to know the different substances which belonged to the animals in order to not to be confusing with the free cefotaxime throughout the study.

Five rats of the groups A and B, were sacrificed after 30, 60, 90, 120, 150, 180 and 210 minutes after the antibiotic administration. The blood, liver, spleen, kidney, lung and heart of those rats were extracted and after that, they were measuring their free cefotaxime concentration by HPLC.

Chemical and Reagents

Cefotaxime sodium was supplied by the Teaching Hospital of Madrid. Vanadium (III) acetylacetonate was obtained from Merck. Trichloracetic acid supplied by Scharlau, this substance was used in concentrations of 10% and 20%.

The mobile phase reagents used were HPLC grade methanol from Scharlau. Water used was from a Milli-Rho-Milli-Q system (Millipore, Bedford, MA, USA). Phosphate buffer (0.1 M) was prepared with phosphate monopotasic anhydride and o-phosphoric acid supplied by Merck.

Apparatus and instrument

Kontron high-pressure liquid chromatograph equipped with a Kontron 420 pump. An automatic injector with 6 valves Kontron Auto Sampler 460. A variable-wavelength UV detector of Kontron Uvikon 735 LC. Phenomenex C_{18} column (25 x 0.46 cm), fulled with particles of 5 μm, besides this column of separation, a precolumn and a protective column fulled with the same material that the first column were used. A Kontron Station Data with D450 software was used to control the detector and injector, on the other hand it allowed the integration of the chromatogram peaks and it cuantificated the results.

ANALYTICAL PROCEDURE

The extraction of cefotaxime in blood was carried out by means of protein precipitants such as trichloroacetic acid (8) in the concentration 10%. 1.5 mL of this reagent was added to 0.5 mL of blood. This mixture was centrifuged at 3000 rpm for 5 min. The supernatant was collected through a Pasteur pipet and filtered through MFS disks of nylon of 0.4 mm of diameter. After that, it was assayed by HPLC.

The extraction of cefotaxime in organs such as liver, spleen, kidney, lung and heart, was carried out by means of trichloroacetic acid 20%. 2 mL of this reagent were added to 0.5 g of dry weight of each organ. Each mixture was done homogeneous and was centrifuged at 3000 rpm for 10 min. The supernatant was collected through a Pasteur pipet and filtered through MFS disks of nylon of 0.4 mm of diameter. It was later assayed by HPLC.

Isocratic HPLC conditions used along this experiment were: the mobile phase consisted of a 20:80% solution (v/v) of methanol in 0.01 M PO_4H_2K. The pH of the final solution was adjusted to 3.2 with phosphoric acid. The flow rate was 1 mL/min. Chromatograph was loaded with 40 µL of each sample. Chromatograms were run at room temperature. Samples were detected at 254 nm.

RESULTS AND DISCUSSION

Concentration of cefotaxime

The concentration homogenates of free cefotaxime was measured in blood, liver, kidney, spleen, lung and heart of the killed rats at the 30, 60, 90, 120, 150, 180 and 210 min. after the administration of cefotaxime, those rats were the rats with vanadium and antibiotic and the rats with cefotaxime only. As there were 5 rats by each time, the average concentration of free cefotaxime was calculated by each group of 5 rats that were killed at each time.

TABLE I. *Average concentrations (mg/mL) of free cefotaxime in rats without vanadium*

	Time (Min)						
Samples	**30**	**60**	**90**	**120**	**150**	**180**	**210**
BLOOD	0.80	0.46	0.25	0.14	0.08	0.06	0.02
LIVER	$6.03.10^{-5}$	$2.68.10^{-5}$	$2.16.10^{-5}$	$1.71.10^{-5}$	$1.42.10^{-5}$	$1.37.10^{-5}$	$1.24.10^{-5}$
SPLEEN	$2.70.10^{-5}$	$1.58.10^{-5}$	$1.45.10^{-5}$	$0.53.10^{-5}$	$0.41.10^{-5}$	$0.39.10^{-5}$	$0.38.10^{-5}$
KIDNEY	$24.2.10^{-5}$	$18.1.10^{-5}$	$10.6.10^{-5}$	$5.90.10^{-5}$	$5.01.10^{-5}$	$4.81.10^{-5}$	$3.17.10^{-5}$
LUNG	$7.19.10^{-5}$	$5.26.10^{-5}$	$3.52.10^{-5}$	$3.02.10^{-5}$	$1.33.10^{-5}$	$1.22.10^{-5}$	$0.46.10^{-5}$
HEART	$2.56.10^{-5}$	$1.87.10^{-5}$	$1.23.10^{-5}$	$1.14.10^{-5}$	$1.08.10^{-5}$	$0.86.10^{-5}$	$0.85.10^{-5}$

In blood and in the rest of organs such as liver, spleen, lung, heart and kidney, we can observe in the Table I that the cefotaxime

concentration in rats without vanadium is lesser in accordance with the time that has elapsed since the administration of the antibiotic. In the Table II, the same case is observed in rats with vanadium, the greatest cefotaxime concentration is at 30 min. after the administration of this cephalosporin, and this concentration decreases since the moment of the administration.

TABLE II. *Average concentrations (mg/mL) of free cefotaxime in rats with vanadium*

	Time (Min)						
Samples	**30**	**60**	**90**	**120**	**150**	**180**	**210**
BLOOD	0.51	0.25	0.18	0.13	0.12	0.02	0.01
LIVER	$4.36.10^{-5}$	$1.72.10^{-5}$	$1.41.10^{-5}$	$1.38.10^{-5}$	$1.30.10^{-5}$	$0.32.10^{-5}$	$0.15.10^{-5}$
SPLEEN	$1.46.10^{-5}$	$1.39.10^{-5}$	$1.38.10^{-5}$	$0.66.10^{-5}$	$0.38.10^{-5}$	$0.37.10^{-5}$	$0.21.10^{-5}$
KIDNEY	$20.6.10^{-5}$	$12.8.10^{-5}$	$7.58.10^{-5}$	$5.42.10^{-5}$	$2.49.10^{-5}$	$2.38.10^{-5}$	$1.36.10^{-5}$
LUNG	$3.86.10^{-5}$	$2.59.10^{-5}$	$1.69.10^{-5}$	$1.59.10^{-5}$	$1.31.10^{-5}$	$0.45.10^{-5}$	$0.23.10^{-5}$
HEART	$1.88.10^{-5}$	$1.68.10^{-5}$	$1.12.10^{-5}$	$1.11.10^{-5}$	$1.04.10^{-5}$	$0.66.10^{-5}$	$0.29.10^{-5}$

Concentrations of cefotaxime, in rats with vanadium and without it, appeared to decline in a biphasic manner. This decrease in the cefotaxime concentration happened in the same way that in adults with a normal renal function (9, 10).

In Table I and II, in all samples of the 2 groups of rats, it could be noticed two phases in the decrease of cefotaxime concentration. An initial phase averages 30-90 min. where the concentration decreased slower.

Cefotaxime concentration does not only change with time, but also changes in accordance with the intoxication with vanadium.

In rats without vanadium the free cefotaxime concentration is greater than the concentration in rats with the metal. This fact happened in blood as well as in the other organs analysed in almost all times what have been described. The differences more significant

have been seen in liver, kidney and lung, where there is more concentration of free cefotaxime in rats without vanadium than in rats with it. In spleen and heart, those differences were less significant, although they are very appreciable. In blood, concentrations of free cefotaxime were greater in rats without vanadium, but the differences between concentrations are not as appreciable as in the others samples that have been analysed. So, the presence of vanadium is an influential factor in the concentration of free cefotaxime.

This observation has been supported with the help of a statistical analysis, here the means of concentrations of free cefotaxime, in blood, liver, spleen, lung, heart and kidney of rats without vanadium and of the rats with it, at 30, 60, 90, 120, 150, 180 and 210 min. after administration of this cephalosporin, have been compared. This comparison has been done by means of a T distribution with $p < 0.05$ (Table III).

TABLE III. *Differences more significant in concentrations of free cefotaxime between rats with vanadium and without it*

Samples	Time (Min)	P	Value of T
	60	0.04	2.40
	90	0.02	2.68
BLOOD	180	$6.84.10^{-4}$	5.35
	60	$7.78.10^{-3}$	3.52
	90	0.04	2.44
LIVER	180	$5.46.10^{-6}$	10.6
	210	$2.48.10^{-9}$	28.5
	30	$1.37.10^{-5}$	9.37
SPLEEN	210	$1.37.10^{-5}$	6.03
	60	0.01	3.24
	90	$5.27.10^{-5}$	7.79
KIDNEY	150	$6.11.10^{-4}$	5.44
	180	$1.22.10^{-5}$	9.52

TABLE III. *Differences more significant in concentrations of free cefotaxime between rats with vanadium and without it* (cont.)

Samples	Time (Min)	P	Value of T
	210	$5.89.10^{-5}$	7.76
	30	$4.03.10^{-6}$	11.04
	60	$8.23.10^{-8}$	18.28
	90	1.10^{-5}	9.77
LUNG	120	$1.44.10^{-4}$	6.75
	180	$4.29.10^{-7}$	14.79
	210	$7.28.10^{-4}$	5.29
	30	0.01	3.01
HEART	180	$7.09.10^{-3}$	3.58
	210	$1.16.10^{-6}$	12.9

Therefore, in all those times is dismissed the nule hypothesis and the averages differences of cefotaxime concentrations were statistically significant. As deduced from these differences between the free cefotaxime concentration in rats with and without vanadium did not happen randomly, so those differences in the concentration could be due to the formation of antibiotic/metal complex, remaining a lesser concentration of free cefotaxime.

Distribution of cefotaxime

Tables I and II show the greatest concentration of cefotaxime in blood. In the assayed organs the concentration of this cephalosporin is decreasing in the sense: kidney > liver > lung > spleen > heart. Therefore cefotaxime is mainly stored in kidney. This fact is same in the 2 groups of rats A and B, and because of this, the metal is not an influential factor in the distribution of cefotaxime.

Stability of cefotaxime

The decrease rate constants of the concentration of free cefotaxime in rats with vanadium and in rats without it are of first-order, according to the best values of correlation coefficient. The absolute values of the logarithmic of the observed constants K are shown in Table IV, where the highest value of log K is in heart in the 2 groups of rats, therefore the cefotaxime is more stable in heart than in the others studied organs.

TABLE IV. *The absolute values of the logarithmic of the observed constant K*

	Rats without vanadium	Rats with vanadium
BLOOD	1.73	1.73
LIVER	2.16	1.79
SPLEEN	1.93	1.93
KIDNEY	1.93	1.85
LUNG	1.86	1.85
HEART	2.16	2.03

In rats without vanadium the stability of cefotaxime in organs is decreasing in the sense: heart and liver > kidney and spleen > lung. In rats with vanadium the stability is: heart > kidney > liver, spleen and lung.

In blood it was observed the lowest stability of cefotaxime in the 2 groups of rats.

Therefore in rats that have been or not intoxicated with vanadium the highest and less stability is at the same site.

It is not only important the presence of vanadium in the concentration, distribution and stability of cefotaxime, but also the presence of the metal results to be crucial in the activity of this cephalosporin because of the decrease of the free cefotaxime concentration due to the metal.

If there were a lower concentration of free cefotaxime, it would not reach the minimum inhibitory concentration to kill the microorganism that cause the infection, so that the activity of cefotaxime will decrease or even the cephalosporin will not have activity in many cases. So, in poisoned patients with vanadium, it is very important to bear in mind this fact when they have to follow a course of treatment with cefotaxime.

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