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Influence of cephalosporins on intestinal enzymatic activity

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The effect of the broad spectrum antibiotics cefaclor, cefadroxil, cephradine, cefatrizine, cephaloglycine and cefroxadine was examined on rat intestinal brush border enzymes, aminopeptidase N (E.C. 3.4.11.2), dipeptidyl peptidase IV (E.C. 3.4.14.5) and alkaline phosphatase (E.C. 1.3.1.3.). All the cephalosporins assayed -except cefaclor- inhibit the aminopeptidase N activity, in an uncompetitive manner. Cefatrizine showed the most important inhibitory effect (52.5 %; $p < 0.001$). Cefaclor and cefadroxil have no effect on the activity of the dipeptidyl peptidase IV, while cephaloglycine and cephradine showed a non competitive type inhibition. In contrast, cefatrizine and cefroxadine showed a competitive inhibition for this enzyme. None of the cephalosporins assayed had any effect on alkaline phosphatase activity.

Key words: Cephalosporins, Digestion, Enzymes.

Interaction between drugs and nutrients is a crucial problem in clinical settings and in the understanding of the gastrointestinal effects of the drugs. Orally administered antibiotics, like cephalosporins, can alter the digestion or the absorption of nutrients. Apart from cutaneous reactions, the most frequent side effects of the cephalosporins are gastrointestinal problems, including abdominal pain, diarrhoea and malabsorption at therapeutic doses (5, 26).

Some antibiotics, one of the most widely used drug groups today, have been shown to produce inhibitory effects on the nutrient absorption level, which could help understand some of the above described effects (3).

Thus ALCALDE *et al.* showed that amoxicillin and tetracycline inhibited either the absorption processes of amino acids like L-leucine (3) or sugars like

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D-galactose (1) both *in vitro* and *in vivo* conditions. Furthermore some cephalosporins, broad spectrum antibiotics, as well as cefatrizine, cephaloglycine (16) and cefroxadine (15) also interact with these absorption processes.

The enzymes involved in this process are located in the enterocyte brush border membrane (23). The goal of the present study, is to analyze the possible effect of these cephalosporins (cephradine, cefaclor, cefadroxil, cephaloglycine, cefroxadine and cefatrizine), which inhibit by intestinal absorption (15,16), on oligopeptidase activity, (aminopeptidase N (E.C. 3.4.11.2) and dipeptidyl peptidase IV (E.C. 3.4.14.5). Furthermore the effect of these oral antibiotics on alkaline phosphatase activity (E.C. 1.3.1.3) is analyzed.

Materials and Methods

Animals.– The equipment, handling and sacrifice of animals were performed according to the European Council Legislation 86/609/EEC for the protection of experimental animals. The study was carried out on male Wistar rats weighing between 180-200 g. The animals were fasted overnight with free access to drinking water.

Chemicals.– All the reagents were of the highest purity commercially available. Cefaclor (Eli Lilly), Cephradine (Squibb), Cefadroxil (Bristol-Myers), Cefroxadine (Ciba-Geygi), Cefatrizine and Cefaloglycine (Antibióticos) were kindly donated by the respective laboratories. The antibiotic concentration used in all the experiments was 2 mg mL^{-1}.

Brush border membrane vesicles preparation.– The vesicles were prepared according to SHIRAZI-BEECHEY method (24) with some modifications. A 30 cm long loop from the jejunum of 7 rats was everted, placed in a buffer (mannitol 100 mM and Tris-HCl 2 mM, pH 7.2) and stirred in a Vibro-mixer (model E-1, Sorvall) at maximun speed for three min. Afterwards the suspension was filtered in a Buchner and $MgCl_2$ was added to achieve a final concentration of 10 mM. The homogenate was ice coldly stirred during 20 min. The suspension was centrifuged at 3,000 x g (0-4 °C) for 15 min. The pellet, resuspended in a buffer with mannitol 100 mM, $MgSO_4$ 0.1 mM and Tris-HCl 10 mM (pH 7.4), was centrifuged twice at 27,000 x g (0-4 °C) for 30 min. The final pellet was resuspended in the required volume of a buffer: mannitol 300 mM, $MgSO_4$ 0.1 mM and Tris-HCl 10 mM (pH 7.4) using a No. 27 gauge needle. The vesicles were stored in liquid nitrogen until they were used. The final concentration of proteins was 5-10 mg mL^{-1}.

Purity of the vesicle preparation.– The purity of brush border membrane vesicles preparation was determined by enzymatic criteria, marker enzyme activity of sucrase (E.C. 3.2.1.48) being measured (6). A 12.5 ± 1.0 fold increase in the activity of brush border membrane sucrase over its mucosal homogenate activity was taken as the criterion for purity.

Alkaline phosphatase activity.– Alkaline phosphatase was measured as described elsewhere (9). Briefly, 0.5 mL of vesicle suspension were added to a 1 mL of a buffer containing 10.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 0.2 mM $ZnCl_2$, 100.0 mM Tris-HCl (pH 9.5). After a 15 min preincubation period at 25 °C, the reaction was initiated by addition of 0.5 mL of 20 mM *p*-nitrophenylphosphate and terminated by the addition of 4 mL of 1.125 M NaOH after an incubation of 15 min at 25 °C. Absorbance was monitored at

400 nm. Enzyme activity is expressed as mmol phosphate released (Pi) min^{-1} (mg protein)$^{-1}$. Enrichment of alkaline phosphatase activity from the homogenate was 10.6 ± 0.9 fold.

Peptidase activity.– Enzymatic activity was measured according to AURICCHIO *et al.* (2). The incubation mixture contained 0.36 mmol substrate (L-glycyl-L-prolyl-β-naphtylamide or L-alanyl-β-naphtylamide), 5 mmol of potassium phosphate buffer, at the optimal pH, and enzyme suspension. Incubation was carried out at 37 °C for 30 min and the amount of β-naphthylamide liberated was determined by fluorescence with λ_{exc} 340 nm and λ_{emis} 410 nm.

Protein was determined by the BRADFORD method (4) with bovine serum albumin as standard.

Values are expressed as mmol substrate hydrolyzed (mg prot)$^{-1}$ min^{-1} and represent the means ± ES. Data were analyzed by ANOVA for comparison between the control and treated groups; differences were considered significant at $p < 0.05$.

Results

Effect of cephalosporins on alkaline phosphatase.– None of the cephalosporins assayed had any effect on alkaline phosphatase activity (control 1.43 ± 0.02 *vs* Cefatrizine 1.422 ± 0.02) and for this reason the transphosphorylation in the brush border is unaffected by these antibiotics.

Interaction of cephalosporins on dipeptidylpeptidase IV activity.– The enzymatic activities hydrolyzing the β-naphthylamides are mainly located in the enterocyte brush border. All these enzymatic activities were purified over 12-fold in the brush border fraction, as compared to the total homogenate. Cefaclor and cefadroxyl have no effect on the activity of the brush border enzyme hydrolyzing L-glycyl-L-prolyl-β-naphtylamide. Cephaloglycine and cephradine showed a non competitive type inhibition with the same affinity as the substrate by the enzyme and different $Vmax_{app}$ (fig. 1A). On the other hand, cefatrizine and cefroxadine showed a competitive inhibition and their $Vmax_{app}$ was 524.3 mmol substrate hydrolyzed (mg prot)$^{-1}$ min^{-1} (fig. 1B).

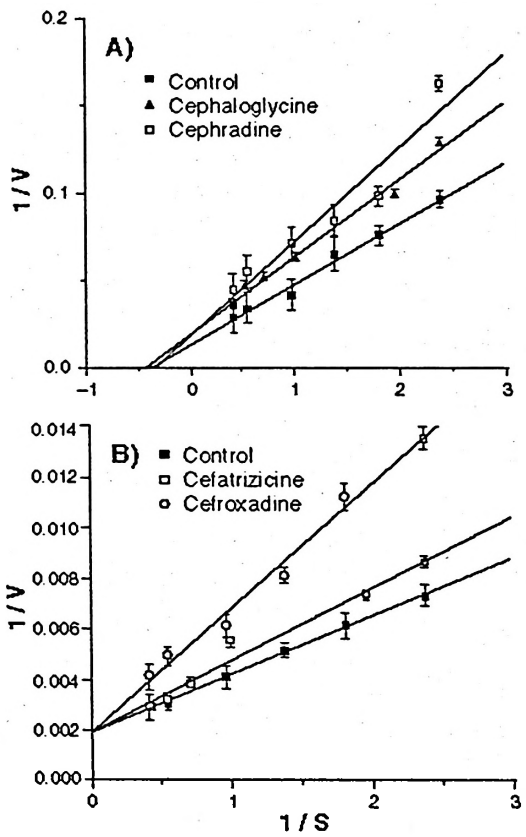

Fig. 1. *Lineweaver-Burk analysis of the inhibitory effect of cephalosporins on dipeptidylpeptidase IV activity*

Six different concentrations of L-glycyl-L-prolyl-β-naphtylamide were assayed to understand the inhibition mechanism. Incubation period 30 min at 37 °C, the concentration of cephalosporins was 2 mg/mL. Values are expressed as mmol substrate hydrolyzed (mg prot)$^{-1}$ min^{-1} and represent the mean ± SE of twelve results.

Table I. *Effect of cephalosporins on aminopeptidase N activity in brush-border membrane vesicles.* Aminopeptidase N activity was measured with different substrate concentrations (mM), the concentration of cephalosporins being 2 mg/mL. The values, (mmol substrate hydrolyzed (mg prot)$^{-1}$ min^{-1}) are the mean ± SE of twelve results from three different membrane preparations. In all the cases $p < 0.001$.

[S]	Control	Cefatrizine	Cephradine	Cefroxadine	Cefadroxil
0.10	204.6 ± 7.5	96.9 ± 3.4	153.0 ± 4.1	144.8 ± 8.1	122.4 ± 6.5
0.55	998.4 ± 8.3	501.3 ± 7.6	761.14 ± 6.2	734.2 ± 7.4	581.8 ± 7.3
0.72	1384.5 ± 9.6	705.1 ± 9.3	1013.1 ± 12.0	1004.8 ± 12.1	784.7 ± 12.2
1.04	2012.1 ± 12.3	976.5 ± 11.3	1490.9 ± 15.2	1507.8 ± 14.4	1166.9 ± 11.3
1.42	2856.1 ± 9.5	1351.7 ± 12.1	2059.1 ± 10.4	2096.3 ± 12.3	1693.6 ± 14.3
1.81	3671.1 ± 17.5	1809.8 ± 12.4	2680.1 ± 11.4	2643.1 ± 11.2	2129.2 ± 9.5

Effect of cephalosporins on aminopeptidase N activity.– All the cephalosporins assayed, except cefaclor, inhibit this dipeptidase in an uncompetitive manner, because the apparent affinity for the substrate of this enzyme and the maxima velocity of the reaction in presence of all the antibiotics decreased (table I). Cefadroxil diminished aminopeptidase activity (42.4 %) in brush border membrane vesicle preparations as did cefroxadine (27.0 %) and cephradine (24.6 %). Cefatrizine showed the most important inhibitory effect on the activity of this peptidase (52.5 %).

Discussion

Cephalosporins are frequently used in human therapeutics as antibiotics (5, 20). They are often orally administered (11) and since they are absorbed in the intestine, there could be drug-nutrient interactions with the digestion and/or absorption processes (17).

Digestion and absorption are highly implicated processes and they reach confluence in the same place, because the digestion final steps occur in the same place where the intestine absorbs the nutrients. Previous studies in our laboratory have shown that these cephalosporins inhibited amino acid absorption (3, 15, 16). Proteolytic enzymatic activity alteration could be added to this effect likely modifying the amino acid, di- or tripeptide production, which would indirectly influence protein absorption.

In this paper the effect of six cephalosporins: cephradine, cefaclor, cefadroxil, cephaloglycine, cefroxadine and cefatrizine on intestinal brush border enzymes, alkaline phosphatase, aminopeptidase N and dipeptidylpeptidase IV has been examined.

Alkaline phosphatase is an almost universal glycoprotein component of mammalian plasma membranes (7). The intestinal brush border is characterized by an intense alkaline phosphatase activity (27). Some substances, present in foods, such as phytic acid (14) and some drugs, such as teophylline (7), decrease alkaline phosphatase activity. Alkaline phosphatase activity remains unaffected by these cephalosporins.

Aminopeptidase N and dipeptidyl peptidase IV have a response to variation in dietary protein content (13, 19, 25) and a diet with low carbohydrate content (18). Dipeptidyl peptidase IV in the small intestine, is located at the brush border membrane of the enterocytes, and this enzyme in kidney and intestine, shares the digestion or reabsorption of peptide that contains proline. The optimal pH values for the hydrolysis in the brush border mem-

brane for different substrates were the following: in Tris-HCl buffer, pH 8 for L-glycyl-L-prolyl-β-naphthylamide and pH 7.5 for L-leucyl-β-naphthylamide (21).

As regards the effects on peptidase activities, the data obtained show that the presence of cefroxadine, cefadroxil, cefatrizine and cephradine decreased aminopeptidase N activity. The fact that aminopeptidase N was inhibited may alter the bioavailability of neutral amino acids, such as L-leucine, which is the product of di- and tripeptide hidrolytic activity. In this case the antibiotics modified the substrate affinity and the apparent Vmax, thus suggesting an uncompetitive type inhibition. The antibiotic binds only to the enzyme-substrate complex for aminopeptidase N. Other authors (13) found that cephalexine caused an uncompetitive type inhibition on aminopeptidase N, although other researchers reported that bestatin inhibits this enzyme in a competitive manner (28). Cefatrizine and cefadroxil were found to produce a large inhibition of aminopeptidase N activity while cefroxadine and cephradine reduced it to a smaller extent. The effect is probably due to the hydroxyl group in their ring structure. This hypothesis is supported as evidenced by the fact that valiolamine, an aminosugar antibiotic with the same structure in its ring, inhibits sucrase activity 1,000 fold greater than its derivates which do not contain this group (12).

Dipeptidyl peptidase IV is attached to the membrane by an amino-terminal anchor, which also functions as a signal peptide (10). In our experiments, dipeptidyl peptidase IV was inhibited by both cefaloglycine and cephradine in a non competitive manner. Cefaclor is well known to have few gastrointestinal side effects. These findings have been confirmed since cefaclor did not produce any changes in enzyme activities.

Our results showed that the inhibition that these cephalosporins produced, except in the case of cefaclor, provoked alterations in protein digestion. This could explain the frequent gastrointestinal side effects produced with treatments using these antibiotics.

Nevertheless this effect is small for total nutrient absorption, due to the rapid turnover (22) of these enzymes in the small intestine.

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Se estudia el efecto de los antibióticos de amplio espectro: cefaclor, cefadroxilo, cefradina, cefatrizina, cefaloglicina y cefroxadina, sobre enzimas del borde en cepillo de intestino de rata: aminopeptidasa N (E.C. 3.4.11.2), dipeptidilpeptidasa IV (E.C. 3.4.14.5) y fosfatasa alcalina (E.C.1.3.1.3.). Todas las cefalosporinas ensayadas, excepto cefaclor, inhiben la actividad de la aminopeptidasa N de forma acompetitiva. La cefatrizina presenta el mayor efecto inhibitorio (52,5 %; $p < 0,001$). El cefaclor y el cefadroxilo no afectan la actividad de la dipeptidilpeptidasa IV, mientras que la cefaloglicina y la cefradina presentan una inhibición no competitiva, y la cefatrizina y la cefroxadina muestran una inhibición competitiva. Ninguna de las cefalosporinas estudiadas tiene efecto sobre la fosfatasa alcalina.

Palabras clave: Cefalosporinas, Digestión, Enzimas.

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