Decreased Aminotransferase Activity of Serum and Various Tissues in the Rat after Cefazolin Treatment

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Treatment of rats with cefazolin in vivo significantly suppressed activity of alanine and aspartate aminotransferases in serum and in the liver, brain, kidney, and heart. Simultaneous administration of pyridoxal further reduced enzyme activity except in the liver, where there was no change. Pyridoxal 5'-phosphate partly reversed the decreased enzyme activity in the serum, liver, and kidney, but did not return it to the amount observed in the control animals; enzyme activity remained suppressed in the brain and heart. The effect of cefazolin was dose related, but there was no sex-related difference. In contrast to its action on aminotransferase activity, cefazolin elicited no effect on alkaline phosphatase (pyridoxal-5'-phosphate hydrodase) in serum or on pyruvate carboxylase in the liver, heart, and kidney. Cefazolin exposed to the hepatic microsomal mixed-function oxidase system in vitro was partly converted into metabolites that inhibited serum alanine aminotransferase activity in vitro. The latter inhibition was reversed by the addition of pyridoxal 5'-phosphate.

Additional Keyphrases: alanine aminotransferase • aspartate aminotransferase • pyridoxal 5'-phosphate • enzyme activity

The recognition and interpretation of adverse effects produced by drugs are important in efforts to prevent these effects. Adverse reactions may alter intracellular metabolism and increase release of cytoplasmic enzymes into the circulation. Among these enzymes, measurement of alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) activity is one of the most important in determining the biochemical profile in the clinical laboratory. Adverse drug side effects are frequently accompanied by increased activities of these enzymes.

The clinical implications of increased aminotransferase values are well known. It is, however, usually difficult to understand the meaning of very low or virtually zero aminotransferase activity. Low results are often found in patients undergoing long-term hemodialysis (1), treated with isoniazid (2), or nutritionally deficient in vitamin B6 (3, 4), and are associated with the loss of pyridoxal 5'-phosphate as a cofactor of aminotransferase (5) by dialysis or by the trapping of the aldehyde by the hydrazide group of isoniazid (6). There are, however, reports that drugs not containing the hydrazide moiety, such as phenothiazines (7) or cefazolin (8), also decrease activity of both aspartate and alanine aminotransferases in humans as well as in experimental animals. In this study we examined the mechanism of action of cefazolin, to establish whether there is any association between its metabolism and its effect on aminotransferase activity.

Materials and Methods

Materials

One gram of Acefa®, the sterile sodium salt of cefazolin ([3-[(5-methyl-1,3,4-thiadiazol-2-yl)thio(methyl)-8-oxo-7-[(1H-tetrazol-1-yl)acetyl]amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid], containing 46 mg of sodium per gram of drug (courtesy of Smith, Kline and French Laboratories, Montreal, Quebec), was dissolved in 3 mL of physiological saline solution and stored at 4 °C. A fresh solution was made up twice weekly. Pyridoxal HCl and pyridoxal 5'-phosphate (Sigma Chemical Co., St. Louis, MO) were dissolved in physiological saline solution and neutralized at concentrations of 200 and 10 g/L, respectively.

Animals

The experiments were conducted with Wistar rats (High Oak Ranch, Richmond Hill, Ontario) kept in an air-conditioned room and given water and food (laboratory chow; Ralston Purina Co., St. Louis, MO) ad libitum. Males weighed 200–250 g, females weighed 80–120 g.

Treatment

Groups were selected without conscious bias. The control group received subcutaneous injections of physiological saline solution, 5 mL/kg of body weight. The cefazolin group received subcutaneous injections of the drug. The cefazolin + pyridoxal group received cefazolin and pyridoxal, the latter being given 1 h before cefazolin. The cefazolin + pyridoxal 5'-phosphate group received cefazolin and pyridoxal 5'-phosphate, the latter being given 1 h before cefazolin. The size of groups in each experiment was equal, ranging from four to 12 animals, and rats of equal weight were always used within one study. All treatments were carried out daily. The test compounds were administered in 5 mL of saline per kilogram of body weight in various doses, as stated in the accompanying figures.

Tissue Samples

At the end of each experiment, blood was withdrawn by cardiac puncture or from the vena cava; after coagulation and separation, the activity of various serum enzymes was determined. Liver, kidney, heart, and brain were removed and tissue homogenates were prepared in physiological saline solution; per liter of solution, the homogenates contained: liver, 10 g; heart, 50 g; and brain and kidney, 100 g each.

From the liver, the post-lysosomal supernate was separated and microsomes were prepared by high-speed centrifugation (9). From other tissue homogenates, the post-lysosomal fraction was obtained by centrifugation at 10 000 X g for 10 min. These fractions were used for enzyme assays, which were

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usually carried out within 24 h after sacrifice. If enzyme determinations were delayed, all serum and tissue fractions were kept frozen at \(-20^\circ C\).

Enzyme Measurements

Determine activities of alanine and aspartate aminotransferases in serum by a modification of the method of Henry et al. (10) in the presence and absence of 10 \(\mu\)g of pyridoxal 5'-phosphate in phosphate buffer (0.1 mol/L, pH 7.4) with AGENT, SGPT, and SGOT test reagent kits (Abbott Laboratories, Diagnostics Division, South Pasadena, CA) and Abbott Bichromatic Analyzer (ABA 100). Measure alkaline phosphatase (EC 3.1.3.1) according to routine laboratory procedures (11), and pyruvate carboxylase (EC 6.4.1.1) by the method of Hager, as modified (12).

Preparation of Cefazolin Metabolites

Add 30–60 mg of cefazolin to 5 mL of an incubation medium containing, per liter, phosphate buffer, pH 7.4, 250 \(\mu\)mol; nicotinamide, 75 \(\mu\)mol; glucose 6-phosphate, 15 \(\mu\)mol; glucose-6-phosphate dehydrogenase, 2 \(\mu\)g (specific activity: about \(1.4 \times 10^5\) U/g); NADP\(^+\), 0.39 \(\mu\)mol; and NAD\(^+\), 0.28 \(\mu\)mol. Shake the medium at 37 \(^\circ\)C in air for 5 min, then start the reaction by adding 1 mL of enzymes from hepatic post-lysosomal or microsomal fractions. Incubate for 60 min. To stop the reaction, insert the reaction flask into boiling water for 10 min. Eliminate denatured proteins by centrifugation, and extract and separate by thin-layer chromatography the supernate, which contains cefazolin metabolites and unchanged cefazolin.

Thin-Layer Chromatography

Separate cefazolin metabolites on Silica gel G type 60 (Brinkmann Instruments, Rexdale, Ontario) by ascending chromatography in a solvent containing n-butanol:glacial acetic acid:water (4/1/2, by vol). Inspect the chromatograms under ultraviolet light and develop them by spraying with 2,7-dichlorofluorescein solution in alcohol (0.5 g/L). For quantitative purposes, the developer can locate a reference cefazolin sample, then scrape off the silica gel from other parts of the thin-layer plate and extract with water. Detect metabolites either with a 10 g/L solution of p-dimethylaminobenzaldehyde dissolved in concentrated HCl/methanol mixture (1/3, by vol); or with an aqueous solution of Fast Blue B salt (5 g/L) followed by 0.1 mol/L NaOH solution; or with sodium \(\beta\)-napthoquinone-4-sulfonate reagent. These reagents are used to identify phenols, amines, or hydrazine-hydrazone derivatives (13).

Statistics and Calculations

These were carried out by the Student’s \(t\)-test (14) on pairs representing control and various treatment groups. Results were considered significant only if \(p < 0.05\).

Results

Administration of cefazolin to male rats significantly reduced the activity of alanine and aspartate aminotransferases in serum three weeks after treatment was begun (Figure 1). Simultaneous administration of pyridoxal in vivo brought about no reversing action on the decreased enzyme activity, and even caused a further reduction. Supplementation with pyridoxal 5'-phosphate was more effective in restoring enzyme activity than pyridoxal was, but still did not increase the amount of enzyme to that of the control animals.

Similarly, cefazolin significantly reduced alanine aminotransferase activity in the liver, brain, kidney, and heart (Figure 2). Parallel treatment with pyridoxal in vivo further diminished the enzyme activity in the brain, kidney, and heart but did not affect activity in the liver. Pyridoxal 5'-phosphate increased alanine aminotransferase activity in vivo in the liver and kidney, as compared to enzyme activities of those tissues in control animals. Aminotransferase activity in the brain and heart was unaffected by pyridoxal 5'-phosphate. In contrast, neither alkaline phosphatase activity in serum nor pyruvate carboxylase activity in various tissues was markedly altered by cefazolin alone or by cefazolin supplemented with pyridoxal or pyridoxal 5'-phosphate in vivo.

When alanine aminotransferase activity of the serum from male or female rats was treated with cefazolin, 1.32 g/L of serum, in vitro for 60 min, enzyme activity remained unchanged. However, when cefazolin metabolites representing the transformation of cefazolin by the liver microsomal mixed-function oxidase system were added to the serum, alanine aminotransferase was significantly suppressed (Figure 3).
3). The decrease showed some time dependence. Addition of pyridoxal 5'-phosphate in vitro increased enzyme activity in both control serum and in serum treated with cefazolin metabolites. However, the amount of increase was greater in samples in which there was reduced enzyme activity brought about by cefazolin metabolites.

After the action of the hepatic microsomal mixed-function oxidase system on cefazolin, the four metabolites were separated, with $R_f$ values of 0.34, 0.54, 0.60, and 0.84. One spot, with an $R_f$ value of 0.54, gave a positive reaction with $p$-dimethylaminobenzaldehyde, which indicated the presence of an amine or a probable hydrazine group.

**Discussion**

These experiments demonstrate that prolonged administration of cefazolin in vivo significantly reduces activity of alanine and aspartate aminotransferases in the serum, liver, brain, kidney, and heart of the rat. The effect could not be reversed by the simultaneous administration of pyridoxal 5'-phosphate when the cefazolin:coenzyme ratio or the cefazolin:coenzyme-precursor ratio was 10:1 by weight. Even when administered in equimolecular doses with cefazolin, pyridoxal 5'-phosphate could not reverse enzyme activity to the amount of activity in the control rats. These results raised the important question of whether the changes in aminotransferase activity in vivo are related to (a) the reduction of pyridoxal 5'-phosphate by a direct action of cefazolin or cefazolin metabolites or (b) a defect specifically developed in pyruvate or 2-oxoglutarate metabolism. It is well documented that iso- niizid and other hydrazines also cause a diminished aminotransferase activity (15, 16). This action is related to hydrazine zone formation, and trapping the coenzyme reduces enzyme activity.

Quantitation of vitamin $B_6$ in tissues and serum revealed that the amount of pyridoxine was unaltered by the administration of cefazolin (17). Moreover, the drug treatment showed no effect on alkaline phosphatase in serum. This enzyme has been shown to govern the control of pyridoxal metabolism in the rat; it functions as pyridoxal-5'-phosphate hydrolase (18). In the organism, pyridoxal 5'-phosphate is bound to protein; when the amount of pyridoxal 5'-phosphate synthetized exceeds the binding capacity of intracellular proteins, the excess free pyridoxal 5'-phosphate is hydrolyzed by hepatic or serum alkaline phosphatase. The unchanged hydrolase activity indicated that cefazolin exerted no action on pyridoxal 5'-phosphate catabolism.

Cefazolin treatment had no effect on tissue pyridoxine content and on the major regulating enzyme of pyridoxal 5'-phosphate metabolism in vivo (simultaneous administration of low amounts of pyridoxal even intensified the effect of cefazolin rather than reversed it), but the pyridoxal 5'-phosphate pool was depleted; therefore, it is probable that the synthesis of pyridoxal 5'-phosphate by kinase was affected by cefazolin. This mechanism of action was confirmed by the finding that there was a difference in the effect on aminotransferase activity in serum and various tissues between the administration of pyridoxal and pyridoxal 5'-phosphate (Figure 1 and 2). On the other hand, cefazolin metabolites may alter aminotransferase activity directly in vitro (Figure 3). The overall effect of cefazolin, therefore, includes an in vitro action on pyridoxal 5'-phosphate synthesis and a partial inhibition of aminotransferase.

The final outcome of cefazolin treatment is that it may exhaust the body pyridoxal 5'-phosphate pool. Many other drugs interfere with the synthesis of pyridoxal 5'-phosphate or with its coenzyme function in essential enzymes. Lack of this compound can lead to many biochemical disturbances (19, 20). Because cefazolin is being applied in therapy, treatment over a prolonged period may cause side effects. Thus, when cefazolin is administered, supplementation with pyridoxine in vivo is probably desirable to maintain the normal activity of enzymes that are dependent upon the presence of coenzymes derived from this compound. Even if the clinical symptoms of pyridoxal deficiency are not apparent, the extensive use of this drug may obscure the evaluation of aminotransferase assays, which are among the most important enzymes used for the diagnosis of a wide variety of diseases (21, 22).

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**References**

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