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Conversion of Ochratoxin C into Ochratoxin A In Vivo

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The conversion of ochratoxin C to ochratoxin A was studied in rats after oral and intravenous administration. The concentration of ochratoxin A in the blood as a function of time was the same after oral administration of equivalent amounts of either ochratoxin C or ochratoxin A. The maximum ochratoxin A concentrations were measured 60 min after administration. Given intravenously, ochratoxin C was also converted to ochratoxin A. Maximum concentrations were reached after 90 min. It is concluded that ochratoxin C is readily converted to ochratoxin A after both oral and intravenous administration. There is reason to believe that a comparable toxicity of the two toxins is based upon this conversion and that only interference with the biotransformation mechanisms may cause a difference in their toxicity.

It is known that among several ochratoxins the most toxic ones are ochratoxin A and its ethyl ester, ochratoxin C (1, 2, 8). Ochratoxin A is frequently found in human food and animal feed in many countries (9). Ochratoxin C rarely occurs as a natural contaminant of these products (9). However, its presence has been demonstrated in feed containing high concentrations of ochratoxin A (5). Galtier and Alvinerie (3) showed that ochratoxin A incubated in vitro with rumen fluid is readily converted to ochratoxin C. The possibility of this transformation occurring in vivo has not yet been resolved. If ochratoxin A is transformed into ochratoxin C, then residual ochratoxin C in food of animal origin may pose sanitation and public health problems (4). Although ochratoxin A is readily absorbed from the intestinal tract (7), the absorption of its ethyl ester, ochratoxin C, has not been demonstrated. Biochemical considerations suggest deesterfication to ochratoxin A before absorption as a possibility, but no details of any biotransformation of ochratoxin C are known.

The aim of this study was to determine the possibility of conversion of ochratoxin C into ochratoxin A after oral or intravenous administration to rats.

MATERIALS AND METHODS

Adult male Wistar rats weighing 250 g from the Institute for Medical Research and Occupational Health colony were used in the experiments. The animals were fed on a standard rat diet (Sljeme; Zagreb, Yugoslavia) and had water and food ad libitum. Twelve hours before administration of ochratoxins, the animals were deprived of food.

For production of ochratoxin A, *Penicillium nordicum* (a kind gift from J. Frisvad) was grown on barley, with moisture adjusted to 30%, using tap water. After 2 weeks at 25°C, the toxin was extracted from the solid with two portions of ethyl acetate. The combined ethyl acetate phases were extracted twice with 0.1 M sodium bicarbonate, which was subsequently acidified with 6 M hydrochloric acid. The water phase was extracted with ethyl acetate. The solvent was evaporated, and the residue was dissolved in chloroform and applied to a silica gel column equilibrated with chloroform. Ochratoxin A was eluted with chloroform and 1% acetic acid. The ochratoxin A-containing fractions were combined and extracted with 0.5 M potassium phosphate buffer, pH 7.5. If necessary, this final product was purified further by silica gel thin-layer preparative chromatography in toluene-dioxane-acetic acid (95:35:4). The toxin was crystallized from benzene and was chromatographically pure and identical with standard ochratoxin A (a kind gift from P. Steyn). Ochratoxin C was prepared by esterfication of ochratoxin A. Ochratoxin A, 4.5 mg, was dissolved in 200 μl of dry ethanol and added to a cold solution of 400 μl of thionyl chloride in 2,000 μl of dry ethanol. After 3 h, the reaction mixture was added drop by drop to an ice-water mixture. The ochratoxin C was extracted with chloroform, and the chloroform was washed with 0.1 M bicarbonate. After evaporation, the residue was dissolved in a small amount of chloroform and purified on a silica gel column equilibrated with hexane. Ochratoxin C was eluted with chloroform and was chromatographically pure. Recovery was 93%. Just before administration, the ochratoxins were dissolved in 1 volume of ethanol and diluted with 9 volumes of 0.5% (wt/vol) sodium bicarbonate. The toxins were given in a volume of 0.5 ml either orally by intubation or intravenously into a tail vein. The doses of ochratoxin A and ochratoxin C were 50 and 53.5 ng/g, respectively, which were equimolar amounts of the toxins.

Ochratoxin A concentration in samples of plasma was measured either by an enzymic method, using 2-ml samples (6), or by a micromethod, using 5-μl samples (K. Hult, R. Fuchs, S. Čeović, M. Peraica, and R. Pleština, J. Appl. Toxicol., in press). For the enzymic method, using carboxypeptidase A, animals were killed at given times with coal gas, and blood was taken from the heart with a heparinized syringe. Plasma was separated by centrifugation. In some animals, the jugular vein was cannulated under ether anesthesia. From these animals, samples were taken repeatedly into heparinized glass capillaries, and plasma was separated before analysis with the micromethod. The micromethod is based on fluorescence measurements in a flow injection system after a simple extraction procedure similar to that used in the enzymic method.

In preliminary experiments, ochratoxin A was measured only by the enzymic method. Later, to follow the dynamics of the conversion in each animal separately, the micromethod was used. Since the two methods gave similar results,

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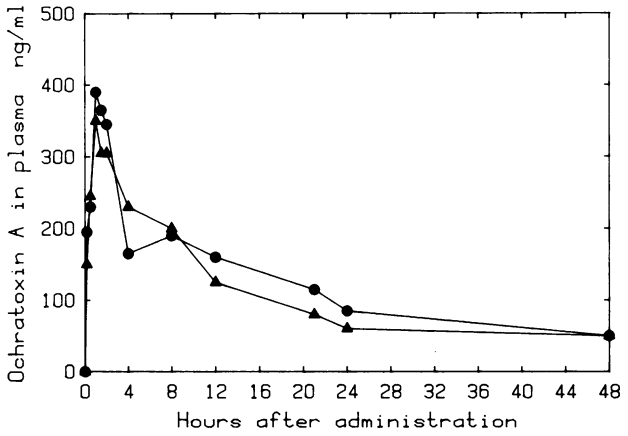

FIG. 1. The concentration of ochratoxin A in plasma after oral administration of ochratoxin A (●) or ochratoxin C (▲) to rats. Doses of the two toxins were equimolar and corresponded to 50 ng of ochratoxin A per g of body weight.

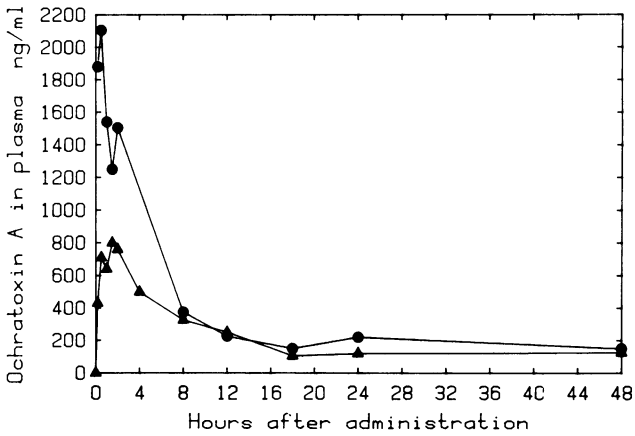

FIG. 2. The concentration of ochratoxin A in plasma after intravenous administration of ochratoxin A (●) or ochratoxin C (▲) to rats. Doses of the two toxins were equimolar and corresponded to 50 ng of ochratoxin A per g of body weight.

these were combined and plotted as means of two to four animals. Altogether, 64 rats were used in the experiments.

RESULTS AND DISCUSSION

After oral administration of either ochratoxin A or ochratoxin C, the highest concentration of ochratoxin A in blood was found after 60 min (Fig. 1). The maximum concentrations found were 390 ng/ml of plasma in rats given ochratoxin A and 350 ng/ml of plasma in rats given ochratoxin C. After 48 h, the concentration of ochratoxin A in both groups was 50 ng/ml.

Intravenous administration of an identical dose of ochratoxin A gave after 30 min a concentration of 2,100 ng/ml of plasma, which fell to 150 ng/ml after 48 h (Fig. 2). However, the level of ochratoxin A in the blood of rats which received ochratoxin C intravenously rose gradually, reaching the highest concentration, 800 ng/ml, 90 min after dosing and thereafter decreased slowly to 130 ng/ml after 48 h.

It appears that the conversion of ochratoxin C into ochratoxin A was very fast after oral or intravenous application. The rate of appearance of ochratoxin A in the blood was exactly the same after oral administration of either ochratoxin A or ochratoxin C. This suggests that the rate of ochratoxin C conversion to ochratoxin A is equal to or faster than the rate of absorption of ochratoxin A from the gastrointestinal tract (Fig. 1 and 2). After intravenous administration of ochratoxin C, the peak appearance of ochratoxin A, which presumably reflects the matching of the rate of conversion of ochratoxin C and the rate of elimination of ochratoxin A, occurred after 90 min.

The ochratoxin A concentration in the blood was almost the same 24 h after administration of either mycotoxin by either route. The elimination of the toxin from the blood during the next 24 h was very slow.

From our results, it is obvious that ochratoxin C is converted to ochratoxin A in vivo. However, it is not clear whether the hydrolysis takes place only in the blood, in which numerous esterases are very active, or also in the gastrointestinal tract. It is known that chymotrypsin readily hydrolyzes the ester bond in ochratoxin C (K. Hult, unpublished data) and that this enzyme is very active in the duodenum, which suggests that hydrolysis to ochratoxin A occurs there after oral intake. The very fast conversion of ochratoxin C into ochratoxin A is a possible explanation of the similar toxicity of the two toxins.

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