Biochemical Pharmacology, Vol. 28. pp. 301–303.
Pergamon Press Ltd. 1979. Printed in Great Britain.

EFFECT OF ANTIBIOTICS ON RAT KIDNEY KYNURENINE AMINOTRANSFERASE ACTIVITY

G. ALLEGRI, C. COSTA and A. DE ANTONI
Institute of Pharmaceutical Chemistry, Padova University, Padova, Italy

(*Received* 20 *February* 1978; *accepted* 6 *June* 1978)

Abstract—The effects of 26 antibiotics on the activity of rat kidney kynurenine aminotransferase were examined.

Among the antibiotics tested, only D-cycloserine and novobiocin showed an inhibiting property on the enzymatic transamination of L-kynurenine and L-3-hydroxykynurenine.

Kinetic studies showed that the type of inhibition is noncompetitive in both cases.

The inhibition constants (K_i) of D-cycloserine with L-kynurenine and L-3-hydroxykynurenine as substrates are 0.25 mM and 0.35 mM respectively, whereas for novobiocin they are 1.29 mM and 1.44 mM respectively.

The K_i values show that D-cycloserine is a potent *in vitro* inhibitor of kynurenine aminotransferase at concentrations similar to those reached *in vivo* in human blood during administration of the drug in therapy.

There are few studies concerning the action of antibiotics upon the kynurenine pathway of tryptophan metabolism. Most of these studies are based upon experiments concerned especially with the induction and inhibition of tryptophan pyrrolase.

The purpose of the present study, therefore, has been to investigate the influence of antibiotics on another enzyme involved in tryptophan degradation: kynurenine aminotransferase (EC 2.6.1.7).

This enzyme catalyzes the conversion of L-kynurenine to kynurenic acid and of L-3-hydroxykynurenine to xanthurenic acid: so both kynurenines were used as substrates.

MATERIALS AND METHODS

L-kynurenine and L-3-hydroxykynurenine were obtained from Calbiochem; 2-oxoglutarate and pyridoxal phosphate from Fluka. Antibiotics D-cycloserine, chloramphenicol, penicillin G, gramicidin D, tyrocidin HCl, bacitracin, streptomycin sulfate, dihydrostreptomycin sulfate, kanamycin sulfate, erythromycin, aureomycin, mycostatin and actidione were purchased from Serva; ampicillin and tetracycline HCl from Farmitalia; polymixin B sulfate and novobiocin sodium salt from Prodotti Gianni; cephaloridine, cephalothin, cephalexine, neomycin sulfate and griseofulvin were given by Glaxo; cephazolin by Carlo Erba; rifampicin by Lepetit; and lyncomycin and clindamycin by Upjohn.

Kidneys from adult male rats weighing about 200 g were used as a source for the preparation of kynurenine aminotransferase, which was partially purified as described by us previously [1], homogenizing the kidneys with 20 mM potassium phosphate buffer, pH 7.2. All procedures were carried out at about 4°. The supernatant was fractionated by using ammonium sulfate. The 70 per cent saturation fraction, after dialyzing overnight, was ultrafiltered with Amicon XM −100 Diaflo membrane and then applied to a column (2.6 × 100 cm) of Sephadex G-150 equilibrated with 20 mM buffer. The active fraction was chromatographed on a DEAE-Sephadex A-50 column (2.6 × 40 cm), and eluted with 300 ml of the same buffer and then with linear gradient between 300 ml volumes of 0.02 M and 0.12 M potassium phosphate buffer, pH 7.2.

Active fractions were pooled, ultrafiltered and applied to a second Sephadex G-150 column eluted with 20 mM buffer, pH 7.2.

A final purification of about 140 times was obtained. The enzyme does not show any activity without added pyridoxal phosphate.

Enzyme assays were performed at 37° according to Mason's method [2] in a total volume of 1.5 ml containing 10.0 mM 2-oxo glutarate, 40.0 μM pyridoxal phosphate, 1.0 mM L-kynurenine (or L-3-hydroxykynurenine) in 50 mM potassium phosphate buffer, pH 7.2 (final concentrations) and the antibiotic when this substance was used. The antibiotic concentration ranged from 1.0 μM to 100 mM when possible. The reaction was stopped at zero time for the blanks and after 30 min by adding 7.5 ml of 1% boric acid in ethanol.

The enzyme activity was measured spectrophotometrically by the change in absorbance at 333 nm for kynurenic acid (from L-kynurenine as a substrate), and at 328 nm for xanthurenic acid (from L-3-hydroxykynurenine).

When the antibiotic was active, as for novobiocin and D-cycloserine, kinetic studies were carried out varying the concentration of the substrate (L-kynurenine or L-3-hydroxykynurenine) and of the pyridoxal phosphate in the absence and in the presence of a fixed amount of antibiotic; on the other hand, the concentration of 2-oxoglutarate was kept at saturation levels (10.0 mM in the assay).

When high concentrations of substrate were used, in many cases it was necessary to determine the kynurenic or xanthurenic acids formed as previously

reported [3], by eluting their spots after bidimensional paper chromatography on Whatman No. 1 of 0.5 ml of the solution used for spectrophotometric analysis.

The affinity of the enzyme to the substrate, and to the inhibitors was determined according to the method of Lineweaver and Burk [4].

RESULTS AND DISCUSSION

Most of the 26 antibiotics reported in "Materials and Methods". tested on the activity of rat kidney kynurenine aminotransferase, using two different substrates L-kynurenine and L-3-hydroxykynurenine, indicated no appreciable effect under our assay conditions, with the exception of D-cycloserine and novobiocin. These two antibiotics showed an inhibiting property on the enzyme.

The inhibition of some transaminating enzymes by L- and D-cycloserine is known, but the inhibition of kynurenine aminotransferase with this drug does not seem to have been reported previously. Wong *et al.* [5] studying the effects of L- and D-cycloserine on rat liver alanine-, phenylalanine- and tyrosine aminotransferases observed a lower potency of L-cycloserine in blocking phenylalanine aminotransferase and tyrosine aminotransferase activities with respect to alanine aminotransferase with which complete inhibition by L-cycloserine was achieved at 10^{-5} M concentration. The inhibition was irreversible and non-competitive with respect to the amino acid substrate. The D-cycloserine was less active in all three cases. Tyrosine aminotransferase was the enzyme least sensitive to inhibition by cycloserine.

Our findings show that D-cycloserine has the same inhibiting effect on rat kidney kynurenine aminotransferase with both L-kynurenine and L-3-hydroxykynurenine as substrates. About 0.3 mM D-cycloserine in the assay was required to attain 50 per cent inhibition. Under the same conditions, novobiocin is about 10 per cent inhibitory. While D-cycloserine at the same concentration as the substrate in the assay (1.0 mM) reduced the activity of this enzyme by about 85 per cent, at this concentration novobiocin caused a 40 per cent inhibition.

The mechanism of inhibition as estimated graphically at different concentrations of the substrates or pyridoxal phosphate, has been studied extensively with both antibiotics. Figures 1a and 1b show double reciprocal plots of the rates of kynurenic acid (Fig. 1a) or xanthurenic acid (Fig. 1b) formation as a function of concentration of L-kynurenine or L-3-hydroxykynurenine in the presence and in the absence of 0.4 mM D-cycloserine or 1.0 mM novobiocin. Inhibition was of non-competitive type for both antibiotics with both substrates, with a constant reduction in V_{max} and no change in apparent K_m.

The estimated K_i values for D-cycloserine and novobiocin were 0.25 mM and 1.29 mM, respectively, with L-kynurenine as a substrate, and 0.35 mM and

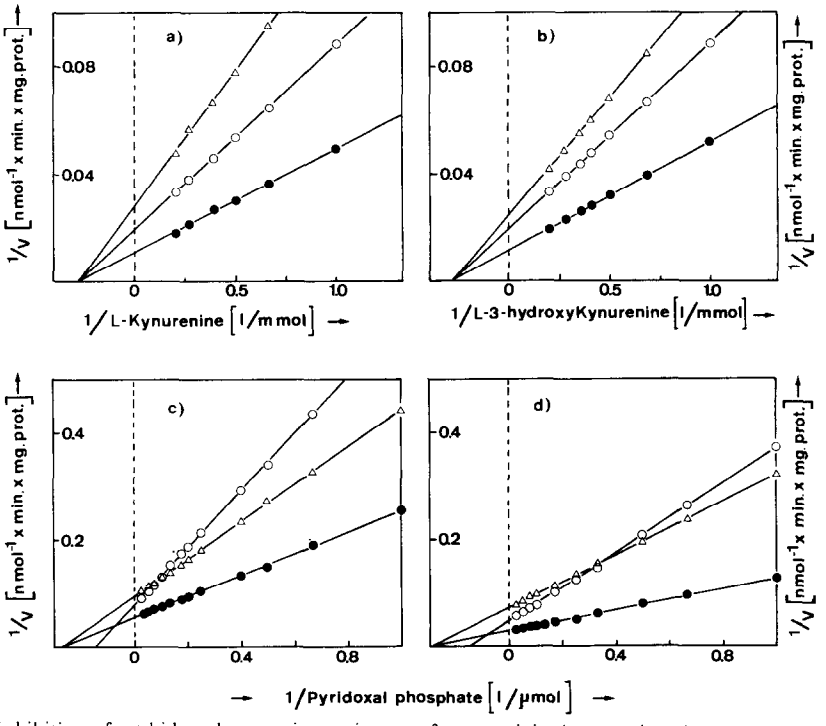

Fig. 1. Inhibition of rat kidney kynurenine aminotransferase activity by D-cycloserine and novobiocin. (a) with L-kynurenine as a substrate at saturation levels of pyridoxal phosphate (40 μM in the assay). (b) with L-3-hydroxykynurenine as a substrate at saturation levels of pyridoxal phosphate (40 μM in the assay). (c) with varying levels of pyridoxal phosphate with 1.0 mM L-kynurenine in the assay. (d) with varying levels of pyridoxal phosphate with 2.0 mM L-3-hydroxykynurenine in the assay.

——●—— no antibiotic
——○—— with 1.0 mM novobiocin
——△—— with 0.4 mM D-cycloserine in a, b, c, and 0.6 mM in d.

1.44 mM respectively with L-3-hydroxykynurenine. The variations in K_i observed with the two different substrates are probably due to experimental errors, and the nature of the inhibition must be considered as tentative.

Figures 1c and 1d show double reciprocal plots of the rates of kynurenic acid (Fig. 1c) or xanthurenic acid (Fig. 1d) formation as a function of concentration of pyridoxal phosphate in the presence and in the absence of 0.4 mM (Fig. 1c) or 0.6 mM (Fig. 1d) D-cycloserine or 1.0 mM novobiocin. Inhibition was of non-competitive type for D-cycloserine and of mixed type for novobiocin. The values of K_i were calculated to be approximately 0.55 mM for D-cycloserine and 1.88 mM for novobiocin, when L-kynurenine was used as a substrate and 0.39 mM for D-cycloserine and 1.23 mM for novobiocin with L-3-hydroxykynurenine.

These results indicate that D-cycloserine is a potent *in vitro* inhibitor of kynurenine aminotransferase and that novobiocin is considerably less effective as an inhibitor. In fact, the calculated K_i values for novobiocin were about 5 times higher than those showed by D-cycloserine.

It is worthy of attention that the K_i values observed for D-cycloserine are similar to the concentrations obtained in the blood and about 5 times slower than those observed in the urine after oral administration of the antibiotic in man [6].

Our findings indicate that D-cycloserine may interfere with tryptophan metabolism, by altering the response to the excretion of certain metabolites. In fact, Vitek *et al.* [7] have observed that in man all forms of cycloserine interfere with the metabolism of tryptophan, increasing the excretion of 5-hydroxyindoleacetic acid and most markedly that of indoleacetic acid.

But D-cycloserine, inhibiting kynurenine aminotransferase activity, can alter the enzymatic conversion of L-kynurenine and L-3-hydroxykynurenine. These two metabolites could accumulate or be transformed into anthranilic acid and 3-hydroxyanthranilic acid, respectively, by means of kynureninase.

3-Hydroxyanthranilic acid and 3-hydroxykynurenine are known to induce neoplasms under certain conditions [8, 9]. On the other hand, it should be mentioned that Musajo [10] found a high urinary excretion of 3-hydroxyanthranilic acid in patients with pulmonary tuberculosis, in which disease D-cycloserine is administered when an antibiotic of the streptomycin group cannot be used because the organism is resistant or the patient hypersensitive.

The K_i values for novobiocin are on the other hand considerably greater than those obtained in blood and in urine after administration of the drug [11, 12], therefore inhibiting effects of novobiocin on kynurenine aminotransferase *in vivo* are hypothesizable only with difficulty.

In conclusion, it seems reasonable to assume that the activity of some antibiotics may lead to the maintenance of an abnormally high concentration of 3-hydroxykynurenine and 3-hydroxyanthranilic acid in the organism. Thus, their effects on tryptophan metabolism should not be undervalued.

REFERENCES

1. L. Musajo, G. Allegri, A. De Antoni and C. Costa, *Acta Vitam. Enzymol. Milano* **29**, 318 (1975).
2. M. Mason, *J. biol. Chem.* **227**, 61 (1957).
3. C. A. Benassi, F. M. Veronese and A. De Antoni, *Clinica chim. Acta* **17**, 383 (1967).
4. H. Lineweaver and D. Burk, *J. Am. chem. Soc.* **56**, 658 (1934).
5. D. T. Wong, R. W. Fuller and B. B. Molloy, *Adv. Enzyme Reg.* **11**, 139 (1973).
6. R. F. Morton, M. H. McKenna and E. Charles, *Antibiotics A.* 169 (1955–56).
7. V. Vitek, K. Rysanek, Z. Horakova, J. Muratova, M. Vojtechovsky and R. Vejdovsky, *Čas. Lék. cesk.* **104**, 113 (1965); *Chem. Abs.* **63**, 6179a (1965).
8. M. J. Allen, E. Boyland, C. E. Dukes, E. S. Horning and J. G. Watson, *Br. J. Cancer* **11**, 212 (1957).
9. G. T. Bryan, R. R. Brown and J. M. Price, *Cancer Res.* **24**, 596 (1964).
10. L. Musajo, A. Spada and D. Coppini, *J. biol. Chem.* **196**, 185 (1952).
11. R. Martin, Y. Chabbert and B. Sureau, *Presse Méd.* **64**, 1597 (1956).
12. H. J. Simon, R. M. McCune, P. A. Dineen and D. E. Gogers, *Antibiot. Med. clin. Ther.* **2**, 205 (1956).