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SHORT COMMUNICATIONS

Inhibition of diamine oxidase of rat small intestine by pentamidine and berenil (diminazene aceturate)

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Polyamines putrescine, spermidine and spermine are considered to be essential for normal cell growth and division and much effort has been made to find specific inhibitors of polyamine biosynthesis [1, 2]. One of the most widely studied inhibitors is methylglyoxal bis(guanylhydrazone) (MGBG; Fig. 1), which is a well-known cytotoxic agent and a potent inhibitor of eukaryotic *S*-adenosyl-L-methionine decarboxylase (ADC), the rate-limiting enzyme in spermidine and spermine biosynthesis [3, 4]. MGBG also affects other enzymes in polyamine metabolism [5, 6] and especially it is a strong non-competitive inhibitor of diamine oxidase inhibiting the enzyme both *in vitro* and *in vivo* [5, 7].

Pentamidine (4,4′-diamidinodiphenoxypentane; Fig. 1) is a drug that is effective in therapy for *Pneumocystis carinii* pneumonia [8] and berenil (4,4′-diamidinodiazoaminobenzene; diminazene aceturate; Fig. 1) an agent with trypanocidal and babesicidal activity [9]. The biochemical mechanism of action of these drugs is so far poorly understood but is supposed to partly be due to interference with DNA synthesis [8, 9]. However, recently it was shown by us [10] and later confirmed by others [11, 12] that both pentamidine and berenil, which are aromatic derivatives of MGBG, inhibit putrescine-stimulated ADC. In the present paper pentamidine and berenil as well as some of their congeners are shown to be also powerful inhibitors of diamine oxidase.

Materials and methods

Chemicals. (1,4-^{14}C)Putrescine dihydrochloride (sp. act. 11.3 mCi/mmol) was purchased from New England Nuclear Corp. (Dreieichenhain, F.R.G.). Berenil was obtained from Hoechst. A.G. (Frankfurt-am-Main, F.R.G.). Pentamidine, propamidine (4,4′-diamidinodiphenoxypropane; Fig. 1) and 4,4′-diamidinodiphenylamine (Fig. 1) were generously given by May and Baker (Dagenham, Essex, U.K.). MGBG was obtained from Aldrich Chemical Co. (Milwaukee, WI) and MBAG (1,1′((methylethane-diylidene)-dinitrilo)bis(3-aminoguanidine); Fig. 1) was synthesized as described earlier [13]. All other biochemical reagents were products of Sigma Chemical Co. (St. Louis, MO).

Enzyme preparation and assay. Diamine oxidase was purified from rat small intestine by ammonium sulphate fractionation and DEAE-Sephadex chromatography by the method of Mondovi *et al.* [14]. The activity of diamine oxidase was assayed as described by Tryding and Willert [15].

Results and discussion

Table 1 demonstrates the inhibition of diamine oxidase of rat small intestine by pentamidine, berenil and some of their structural analogues. Both pentamidine and berenil appeared to be potent inhibitors of the enzyme since inhibitor concentrations of only 3 μM and 0.3 μM were needed for 50% reduction in enzyme activity, respectively. Propamidine and 4,4′-diamidinodiphenylamine, whose structure closely resembles that of pentamidine and berenil, also powerfully inhibited diamine oxidase activity at micromolar

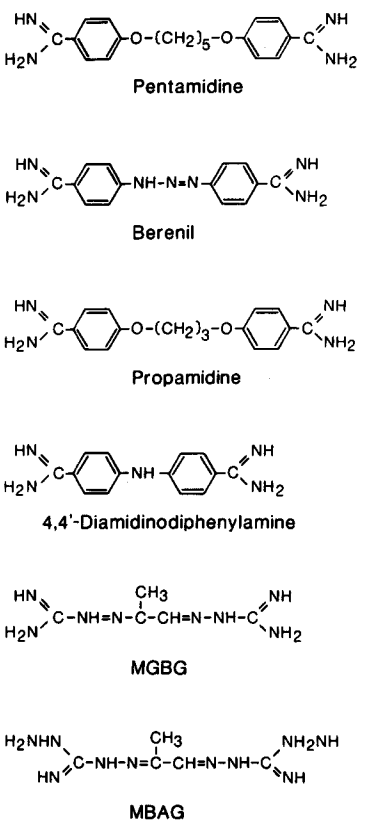

Fig. 1. Structures of compounds used in this study.

Table 1. Pentamidine and its structural analogues as inhibitors of diamine oxidase

Inhibitor	Concentration (μM) needed for 50% inhibition
Pentamidine	3
Berenil	0.3
Propamidine	5
4,4′-Diamidinodiphenylamine	0.3
MGBG	0.4
MBAG	0.04

Diamine oxidase from rat small intestine was purified and assayed as described in Materials and Methods.

concentrations (Table 1). The degree of inhibition of the enzyme by MGBG and MBAG, which were used in the study as controls, was of the same order of magnitude as reported earlier [5, 7].

The type of inhibition of diamine oxidase activity by pentamidine and berenil was studied more detailed (Figs 2A and 2B). Both pentamidine and berenil were found to be non-competitive inhibitors of the enzyme with K_i values of 3.8 μM and 0.4 μM, respectively. MGBG, also a non-competitive inhibitor of diamine oxidase, has a K_i value of 0.1–0.7 μM [5, 7], which is thus quite similar to the inhibition constant of berenil whereas pentadine appears to be slightly weaker inhibitor of the enzyme.

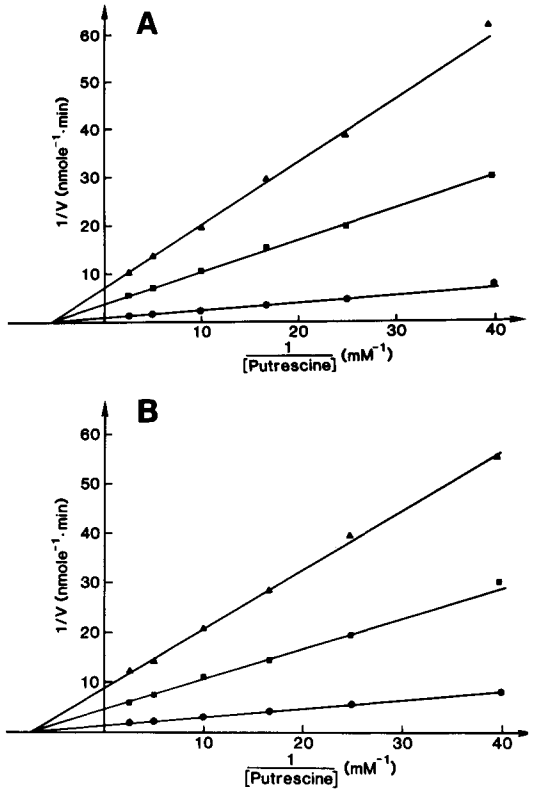

Fig. 2. Kinetics of inhibition of diamine oxidase by (A) pentamidine and (B) berenil. The substrate concentration in the assay medium was varied from 0.025 mM to 0.4 mM: (A) ●, control; ■, 10 μM; and ▲, 20 μM pentamidine; (B) ●, control; ■, 1 μM; and ▲, 2.5 μM berenil.

A recent report by Balana-Fouce *et al.* [11], which appeared after completion of this study, also describes the inhibition of diamine oxidase by berenil. Using a crude enzyme extract from rat small intestine they obtained 50% reduction in enzyme activity with 0.9 μM inhibitor concentration, which is slightly higher than our value (0.3 μM). Furthermore, they reported berenil to be an uncompetitive inhibitor of diamine oxidase. This, however, is in disagreement with our results which indicate the type of inhibition to be non-competitive. The reason for the discrepancy between the results is not clear but it may be due to the crude enzyme preparation used by Balana-Fouce *et al.* Such enzyme extract contains for instance more nucleic acids that can bind berenil [9] thus possibly leading to a different type of kinetics of inhibition of diamine oxidase by the drug.

Both clinically used drugs, pentamidine and berenil, were found to inhibit diamine oxidase at considerably low concentrations and at least pentamidine on therapeutic level (plasma levels 0.5–2.4 μM [8]). This fact should be taken into account when evaluating the physiological effects and mechanisms of action of these drugs in therapy for *Pneumocystis carinii* pneumonia and infections caused by trypanosomes.

In conclusion, the results of this study showed that pentamidine and berenil were potent inhibitors of diamine oxidase *in vitro*. Both pentamidine and berenil appeared to inhibit the enzyme non-competitively with K_i values of 3.8 μM and 0.4 μM, respectively.

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