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Inhibition of enzymes of polyamine back-conversion by pentamidine and berenil

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Abstract—Pentamidine and berenil, clinical antiparasitic amidines, have been found to be potent competitive inhibitors of human spermidine/spermine acetyltransferase (SSAT). K_i values were found to be 2.4 and 2 μM, respectively, with spermidine as substrate. A second enzyme of polyamine back-conversion, murine polyamine oxidase (PAO), was found to be competitively inhibited by pentamidine, with a K_i of 7.6 μM when *N*-acetylspermine was the substrate. Berenil, on the other hand, was an extremely weak inhibitor (K_i = 120 μM). The implication of the effect of inhibition of polyamine back-conversion on the growth of mammalian parasites is discussed.

In addition to the well-known ability of certain amidine compounds to affect growth of mammalian parasites such as *Trypanosomas brucei, T. cruzi*, and *Pneumocystis carinii* through cleavage and inhibition of synthesis of kinetoplast DNA [1, 2], recent work has demonstrated that these compounds also profoundly affect various aspects of polyamine metabolism. Polyamines prevent the curative effect of treatment with amicarbalide and imidocarb on *T. brucei* infections in mice [3]. In fact, polyamine depletion by α-difluoromethylornithine (DFMO), an ornithine decarboxylase (ODC*) inhibitor, cures *T. brucei* infections of mice [4]. Several authors have studied the effects of pentamidine and/or berenil on *S*-adenosylmethionine decarboxylase (AdoMetDC) from either rat liver or *T. brucei*. Balana-Fouce *et al.* [5] found that inhibition of the liver enzyme was competitive, whereas Karvonen [6] reported that inhibition of AdoMetDC is irreversible but did not characterize the inhibition kinetically. Bitonti *et al.* [7] studied the effects of berenil and pentamidine on *T. brucei* AdoMetDC and reported irreversible enzyme inhibition by berenil and reversible inhibition by pentamidine.

In addition to studies on AdoMetDC, both Balana-Fouce *et al.* [5] and Karvonen [6] have examined the effects of amidines on diamine oxidase, an intracellular enzyme which metabolizes putrescine. Balana-Fouce *et al.* found that berenil is an uncompetitive inhibitor of diamine oxidase while Karvonen reported that both berenil and pentamidine are non-competitive. Duch *et al.* [8] demonstrated potent inhibition of rat caecum diamine oxidase by berenil and pentamidine, but kinetic analysis of the inhibition was not reported.

Two additional intracellular enzymes of polyamine catabolism are spermidine/spermine acetyltransferase (SSAT) [9, 10] and polyamine oxidase (PAO) [11, 12]. SSAT and PAO operate sequentially to catalyze the back-synthesis of spermidine from spermine, and then of putrescine from spermidine [13]. The importance of this metabolic pathway has been demonstrated both by Shinki *et al.* [14] who found that the major source of newly synthesized putrescine in cholecalciferol-stimulated chick intestine is derived through this "back-conversion" pathway and by Sato and Fujiwara [15], who demonstrated putrescine production from spermidine in liver after partial hepatectomy. Similarly, Seiler and coworkers [16] have found that a specific PAO inhibitor increases the antiproliferative activity of DFMO by preventing back-conversion of polyamines. Since amidines have been shown to inhibit various other enzymes of polyamine metabolism [5–8], and since the amidines also share certain structural similarities with other inhibitors of SSAT [10], it was of interest to examine the effects of some amidines on the enzymes of polyamine back conversion.

Materials and Methods

Materials. [^{3}H]N^1-Acetylspermine was synthesized as previously reported [12]. [^{14}C]Acetylcoenzyme A (54 mCi/mmol) was purchased from ICN. SSAT was purified from human melanoma cells as described previously [17]; assays were carried out as previously reported [17, 18]. PAO was purified from L1210 extracts as described in Ref. 12, and the A isozyme was used for inhibition studies.

PAO assay. Enzyme was incubated with 5 μmol bicine buffer, pH 8.8, 0.3 μmol dithioerythritol, 33 nmol [^{3}H]-acetylspermine, and inhibitor (when present) for 40 min at 37° with shaking. The reaction was stopped by the addition of 50 μL of 1 N HCl containing 1% phenylhydrazine. After 5 min of further incubation at 37°, the phenylhydrazone of 3-[^{3}H]acetamidopropranal was extracted into 1 mL of ethylacetate and the radioactivity was determined by liquid scintillation counting.

Results and Discussion

Both pentamidine and berenil proved to be potent inhibitors of SSAT. The two compounds were shown to be competitive inhibitors of spermidine acetylation as shown by the Lineweaver–Burk plot (Fig. 1) with similar K_i values, 2.4 and 2.0 μM, respectively. These values are nearly comparable to the inhibition constants for bis-ethyl derivatives of spermine analogs, which are, at present, regarded as the most potent competitive inhibitors of SSAT [17]. N^1,N^{11}-Bis(ethyl)norspermine (BENSPM) and N^1,N^{12}-bis(ethyl)spermine (BESPM) have been reported [17] to have K_i values of 0.8 and 1.9 μM, respectively. In contrast to the very similar inhibitory effects exhibited by pentamidine and berenil against SSAT, there was a large divergence in inhibitory potency against L1210 PAO. While pentamidine was a moderately potent competitive inhibitor of PAO, with a K_i of 7.6 μM, berenil was an extremely weak inhibitor with a K_i value of 120 μM. Figure 2 illustrates the inhibition of *N*-acetylspermine oxidation by these two compounds.

The results suggest that either berenil or pentamidine will inhibit the back-conversion of the higher polyamines spermine and spermidine to putrescine. Since pentamidine potently inhibits both SSAT and PAO, we may anticipate that, in mammalian cells, pentamidine will prevent the oxidation of spermine first to spermidine and then to putrescine. If the production of putrescine by this pathway is of importance for mammalian cells [14, 15], we may anticipate that pentamidine will inhibit their proliferation.

Similar conclusions may be drawn about the effect of berenil, in spite of the fact that it is a rather weak inhibitor of PAO with a K_i of 120 μM. Since berenil can inhibit

* Abbreviations: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine; AdoMetDC, *S*-adenosylmethionine decarboxylase; SSAT, spermidine/spermine acetyltransferase; and PAO, polyamine oxidase.

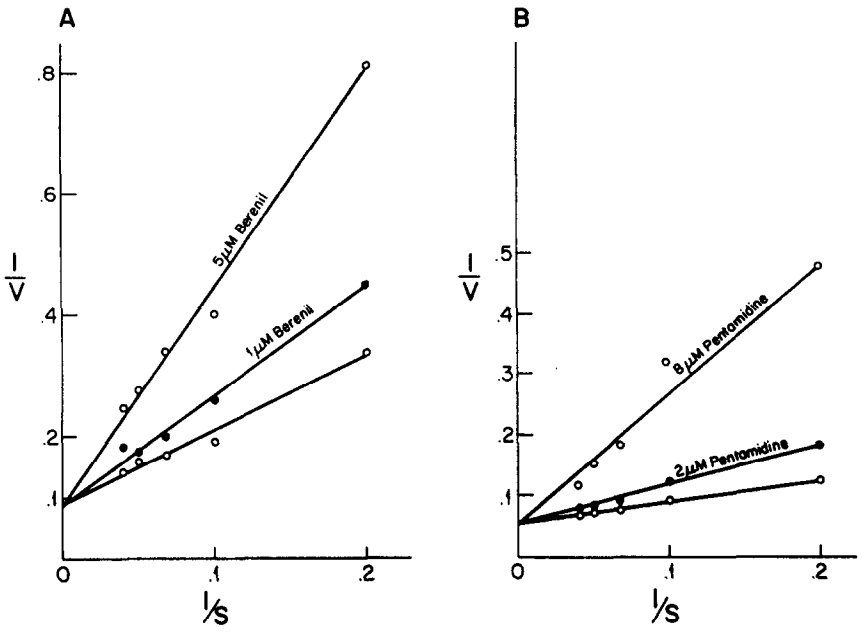

Fig. 1. Inhibition of human melanoma SSAT by berenil (panel A) and pentamidine (panel B).

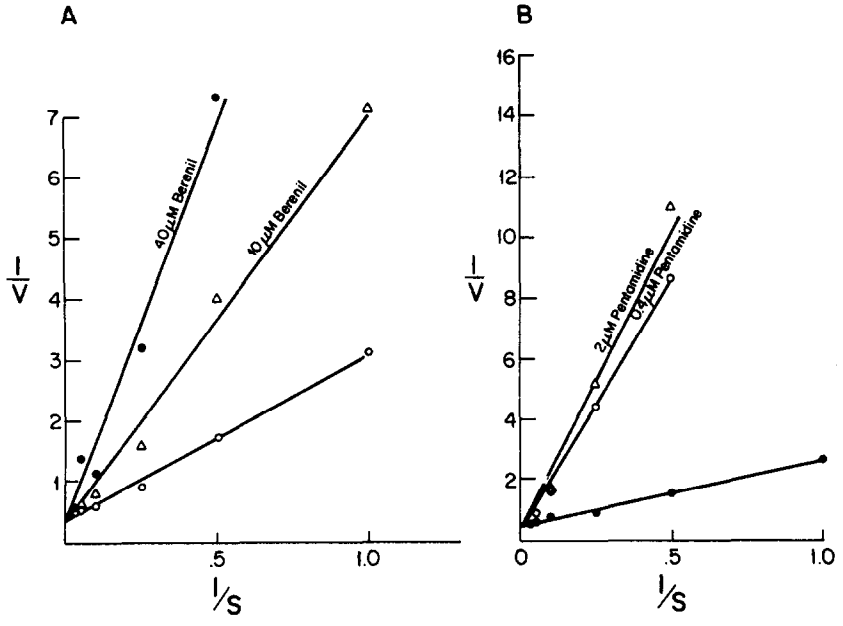

Fig. 2. Inhibition of murine L1210 leukemia PAO-A by berenil (panel A) and pentamidine (panel B).

SSAT, with a K_i of 2 μM we may anticipate a major decrease in the formation of acetylspermine and acetylspermidine. This is of major importance, since mammalian PAO shows 3-fold lower affinity for spermine than for acetylspermine; similarly, the affinity for spermidine is much lower than for acetylspermidine [12]. (The kinetic constants for spermine and spermidine were determined in the presence of optimal concentrations of added benzaldehyde; it is not known if free spermine and spermidine can be oxidized by PAO intracellularly in the absence of added aldehydes.) Although PAO is not strongly inhibited by berenil, we may anticipate a substantial

decrease in polyamine oxidation since berenil inhibits SSAT, the enzyme which forms the preferred substrates for PAO.

We emphasize that these studies were performed with the mammalian enzymes. It is not known whether the actions of pentamidine and berenil on the back-conversion enzymes actually contribute to the known antitrypansomal actions of the two compounds since these drugs have also been shown to affect kinetoplast DNA [1, 2]. *T. brucei* is not known to contain either SSAT or PAO; in addition it lacks spermine [19]. We may hypothesize that treatment of animals infected with parasites such as *T. brucei* or *P. carinii* may have two separate effects which would tend to restrict spermidine availability to the parasite: first, since AdoMetDC is inhibited [5, 7], the conversion of putrescine to spermidine will be halted; and second, since host SSAT and PAO are inhibited by the compounds, the conversion of host spermine to spermidine will be blocked. Thus, the amount of serum spermidine available to the parasite could be restricted. In the case of tumor systems, it has been shown that such restriction enhances the antiproliferative activity of polyamine inhibitors [16]. The actions on both parasite AdoMetDC and host back-conversion may therefore decrease the levels of spermidine available to the parasite to support growth, thus restricting parasite growth; these actions may tend to augment the other, known, actions of berenil and pentamidine on parasitical kinetoplast DNA synthesis and cleavage [1, 2].

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