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SHORT COMMUNICATION

Competitive inhibition of dehydrogenases and kinases by 9-aminoacridine and quinacrine

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Among acridine derivatives are well known mutagenic [1], antitrypanosomal [2] and antitumor [3–5] agents. The inhibitory effect of acridine derivatives on nucleic acid synthesis [6–8] is noteworthy. The antimalarian agent quinacrine [9] has a local anesthetic effect [10] which may be related to its blocking effect on axonal conduction [11]. In fact, quinacrine is a potent inhibitor of acetylcholine transport into purified vesicles [12] and inhibits a number of processes, most notably phospholipase A_2 activity [13], calcium flux [14] and neurotransmitter release [15]. In rat brain slices, quinacrine seems to inhibit primarily the synthesis of acetylcholine. Furthermore, quinacrine accumulates in certain peptide-hormone producing cells [16], in specific regions of the autonomic nerve system [17], in some regions of guinea pig brain [18] and in nerves of the spinal cord [19].

Intercalation of acridine derivatives into DNA [20] seems to trigger the action of topoisomerases, producing cleavage in DNA [21]. Surprisingly, the affinity of 9-aminoacridine by the DNA molecule does not correlate with its mutagenic activity [22], and some acridine derivatives that have a higher relative affinity to DNA [23] lack antitumor effect [3, 24]. Furthermore, the antitumor effects of diacridines, which are extensively taken up by cells in culture [25] do not correlate with the inhibition of DNA or RNA synthesis *in vivo* but seem to be associated with very specific membrane sites that may be primarily characteristic of tumor cells [26]. The uncoupling of oxidative phosphorylation by acridine derivatives [27] may also be related to a primary effect of these compounds on membranes.

From the effects of acridine derivatives on enzymes that use ATP [28, 29] it was deemed reasonable to study if the acridine nucleus could be recognized by the active center of enzymes utilizing adenine(di)nucleotides. In an attempt to gain insight into the possible molecular mechanism(s) by which acridine derivatives manifest their cytotoxicity, we explored the effects of 9-aminoacridine and quinacrine on the activity of a number of enzymes that utilize adenine-(di)nucleotides as substrates.

Materials and methods

Pyruvate kinase and lactate dehydrogenase from rabbit muscle, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase (NADP-linked) and hexokinase from yeast, malate dehydrogenase (NAD-linked) from pig heart, phosphoenol pyruvate, NADH, ADP, Tris-(hydroxymethyl)aminoethane, oxalacetate, NADP, glucose-6-phosphate, 9-aminoacridine and quinacrine were from the Sigma Chemical Co. Cyclic AMP dependent protein kinase was prepared from bovine brain and assayed as described [30]. The pyruvate kinase assay was carried out in a medium (1 ml) containing 8 mM $MgCl_2$, 75 mM KCl, 0.78 mM phosphoenol pyruvate, 0.15 mM NADH, 70 μg lactate dehydrogenase, 0.16 μg pyruvate kinase and different concentrations of ADP. Alcohol dehydrogenase was assayed in a medium (1 ml) containing 200 mM ethanol, 0.30 μg alcohol dehydrogenase and different concentrations of NAD. Glucose-6-phosphate dehydrogenase was assayed in a medium (1 ml) containing 1 mM glucose-6-phosphate, 10 mM $MgCl_2$, 42 ng of the enzyme and different concentrations of NADP. Malate dehydrogenase activity was measured in a medium (1 ml) containing 0.5 mM oxalacetate, 17 ng of enzyme and different concentrations of NADH. The assay medium for hexokinase contained (1 ml) 200 mM glucose, 0.3 mM NADP, 0.6 units glucose-6-phosphate dehydrogenase, 5.4 μg hexokinase and Mg^{2+}-ATP at different concentrations. All the assays were carried out in 50 mM Tris–HCl (pH 8.0), started by addition of the enzyme, and measured at 30° by recording the decrease or increase in extinction at 340 nm for 2 min. The concentration ratios of lactate dehydrogenase to NADH and glucose-6-phosphate dehydrogenase to NADP, for the assay of pyruvate kinase and hexokinase, respectively, were selected so as to ensure maximal activities of the coupling enzymes at the higher concentration of inhibitor tested.

Results and discussion

Fully competitive inhibition of kinase and dehydrogenase activities by 9-aminoacridine and quinacrine was observed by Lineweaver and Burk plots for adenine(di)nucleotides and secondary plots of apparent K_m against inhibitor concentration. Table 1 summarizes the inhibition constants, K_i for the inhibition of several nucleotide-dependent enzymes by acridine derivatives. From the K_i values shown in Table 1 it is clear that quinacrine was a more potent inhibitor than 9-aminoacridine, suggesting that the substituent in N-9 of the quinacrine molecule does not impose steric restriction to the interaction with the enzyme. The effect of the substituent in N-9 on the inhibitory capacity of acridine derivatives is as yet unknown.

The competitive inhibitions reported above suggest that acridine derivatives are recognized by the nucleotide binding domain of enzymes utilizing adenine(di)nucleotides as substrates.

Adenine and acridine derivatives have a moiety composed of unsaturated coplanar rings, albeit of different size. Hence, for acridines to be recognized as "analogues" by the enzymes, the active sites ought to be flexible enough to accommodate them in spite of their size difference to the adenine moiety. Studies on xanthine oxidase and adenosine deaminase [31] established that certain adenine analogs, obtained by insertion of benzene (actually four carbons) into the center of the purine ring system, are substrates of these enzymes. These so-called stretched-out adenine analogs were useful to test the dimensional requirements of enzyme active sites by lateral stretching of the normal substrate [31]. Moreover, X-ray diffraction studies demonstrated that Cibacron blue (an anthraquinone derivative formed by three aromatic rings) binds to the adenine moiety binding site of alcohol dehydrogenase [32]. Thus, stretched-out benzopurine analogs [31] and the anthraquinone Cibacron blue [32] are unambiguous examples of compounds having a moiety composed of three coplanar aromatic rings capable of being recognized by enzymes utilizing adenine(di)nucleotides.

The possibility that the competition of the acridine nucleus with adenine(di)nucleotides for their binding domain on enzymes could contribute to the cytotoxicity manifested by acridine derivatives should be further investigated by determining the dose-dependent effects of these

Table 1. Inhibition constants of kinases and dehydrogenases for the inhibition by 9-aminoacridine and quinacrine

Enzyme	Concentration tested (mM)		K_i (μM)	
	9-AA	Quinacrine	9-AA	Quinacrine
Pyruvate kinase	1.1–2.2	0.3–0.5	250 ± 30	40 ± 3
Alcohol dehydrogenase	0.1–0.3	0.1–0.3	90 ± 7	70 ± 5
Hexokinase	1.4–1.8	0.3–0.5	1300 ± 98	275 ± 25
cAMP-dependent protein kinase	1.0–2.0	0.45–0.9	1500 ± 105	400 ± 38
Malate dehydrogenase	0.2–1.1	0.2–1.1	900 ± 57	200 ± 16
Glucose-6-phosphate dehydrogenase	0.8–1.4	0.6–1.6	500 ± 34	340 ± 29

The enzymes were assayed as described under Materials and Methods. Inhibition constants (K_i) were determined from plots of apparent K_m values versus inhibitor concentrations. Each result represents the mean ± SD of four determinations of K_i.

compounds on the catalytic activities of kinases and dehydrogenases from different tissues.

In summary, we observed that the acridine derivatives 9-aminoacridine and quinacrine were competitive inhibitor of a number of kinases and dehydrogenases, strongly suggesting that the acridine moiety is recognized by adenine-(di)nucleotide utilizing enzymes as an adenine analogue.

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