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Inhibition of rat lung *S*-adenosylmethionine decarboxylase by *N,N'*-dimethyl-4,4'-dipyridyl dichloride (paraquat)

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Polyamines are ubiquitously distributed endogenous compounds that have been associated with the regulation of numerous cellular functions [1–4]. Although simple in structure, these compounds bind to many classes of macromolecules. The binding capabilities of the polyamines appear to be related to the critical intramolecular distance between the nitrogen moieties which are positively charged at physiological pH. Modulation of polyamine synthesis by specific enzyme inhibitors and precursor analogs has shown that cellular polyamine levels are important determinants for RNA synthesis [5], cellular proliferation [2], differentiation [2] and membrane functions [6].

N-substituted 4,4'-dipyridyl analogs represent a class of compounds similar in structure to putrescine having two charged nitrogens separated by an intramolecular distance of approximately 6.9 A [7]. Previous studies have shown that the *N,N'*-dimethyl analog (paraquat), a commonly used herbicide, is actively accumulated into both human and animal lung tissue by a process that is inhibited by the endogenous polyamines [8, 9]. It has been suggested that the lungs contain a specific transport mechanism for polyamines that recognises paraquat. Moreover, several cellular processes thought to be under polyamine regulation [5, 10] are affected by paraquat. These include DNA synthesis [11, 12] and DNA repair [13]. It is conceivable that *N*-substituted 4,4'-dipyridyl analogs may represent a class of polyamine inhibitors.

The biosynthesis of polyamines requires the decarboxylation of *S*-adenosylmethionine to *S*-adenosyl-5'-deoxy-(5')-3-methyl-thiopropylamine. The latter substrate is the donor of a propylamine group to putrescine during the synthesis of spermidine, and to spermidine during the synthesis of spermine. The decarboxylation reaction is catalysed by *S*-adenosylmethionine decarboxylase (SAMDC*: EC 4.1.1.50), a cytosolic enzyme that is dependent upon putrescine for activation [14]. Putrescine decreases the Michaelis constant K_m with little effect on the maximum velocity of SAMDC. These data suggest that putrescine increases the affinity of substrate for the active site on the enzyme.

The principal aim of the present study was to investigate whether paraquat, and its parent compound dipyridyl, bound to the specific polyamine binding site(s) on SAMDC and whether such an interaction modulated the activity of the enzyme. Experiments were undertaken using rat lung tissue since the lungs are the major site of paraquat accumulation and toxicity [8]. The data showed that the herbicide was an effective inhibitor of the enzyme. However, the nature of inhibition suggested that paraquat did not simply displace putrescine from its enzymatic binding site.

Materials and methods

Preparation of tissue. Lungs from male Sprague–Dawley rats (200–250 g) were flushed with 10 ml 0.9% saline and homogenised at 4° in 4 vol. 100 mM phosphate buffer (pH 7.2) containing 0.1 mM ethylenediamine tetraacetic acid and 1 mM dithiothriotol. The tissue was centrifuged at 3000 *g* for 10 min followed by 110,000 *g* for 60 min at 4°. The resulting supernatant was recovered for SAMDC determinations. Protein content was estimated by the Bradford method [15].

Determination of SAMDC activity. Lung supernatant (0.5 ml) was incubated with 1 mM pyridoxal-5-phosphate, 1 mM putrescine, 0.2 μCi ^{14}C-SAM, paraquat (50–1000 μM) and phosphate buffer containing 1 mM dithiothriotol and 0.1 mM EDTA to a final volume of 1 ml. The incubations were carried out in a 20 ml glass scintillation vial at 37° for 60 min with constant shaking. An aliquot (50 μl) of ethanolamine was placed on a small square of filter paper lodged in the cap of the vial. After 60 min, 0.5 ml 6 M HCl was added to each vial which were then shaken for a further 30 min. The vial caps containing the filter paper were transferred to scintillation vials containing 15 ml scintillant (Aquasol) and ^{14}C content determined by scintillation counting. Appropriate blanks containing either boiled tissue or no tissue were concurrently run with the samples.

Initial studies established that the reaction was linear up to 90 min over a protein concentration range of 2–8 mg/ml, SAM concentration range of 1–400 μM and putrescine concentration range of 50–3000 μM.

In some experiments, superoxide dismutase (1 mg/ml), catalase (10 μg/ml) or 1,3-dimethylurea (1 mM) was added to the incubation. Final volume remained at 1 ml.

Kinetic parameters were determined by least squares regression analysis assuming the rate of reaction obeyed

* Abbreviations used: SAMDC, *S*-adenosylmethionine decarboxylase; ODC, ornithine decarboxylase; DFMO, difluoromethylornithine; MGBG, 1,1'-[(methylethanediylidene)dinitrilo]diguanidine.

Michaelis–Menten kinetics. In the case when this assumption was clearly improper (see text), no regression analysis of the data was undertaken.

Chemicals. *S*-adenosyl-L-[methyl-^{14}C] methionine (specific activity = 60 μCi/μmol) was obtained from Amersham (U.S.A.). Dithiothriotol, pyridoxal-5-phosphate, *S*-adenosyl-methionine, paraquat, 4,4′-dipyridyl, putrescine, catalase (118,000 U/mg), superoxide dismutase (2750 U/mg protein) and 1,3-dimethylurea were purchased from Sigma Chemical Co. (U.S.A.). All other reagents were of analytical grade.

Results and discussion

Paraquat demonstrated a dose-related inhibition of rat lung SAMDC (Table 1). In contrast, 4,4′-dipyridyl, the unsubstituted analog of paraquat, caused no significant change in SAMDC activity at concentrations as high as 1 mM. Unlike paraquat, the nitrogen moieties of 4,4′-dipyridyl are uncharged at physiological pH. The possible role of toxic oxygen metabolites in the activation of SAMDC was investigated. Paraquat is capable of undergoing a one-electron reduction to form a cationic radical that can subsequently reduce molecular oxygen to superoxide [16]. Although the reduction of paraquat is most commonly thought to be catalysed by microsomal cytochrome P-450 reductase, cytosolic enzymes such as glutathione reductase can also participate in this reaction [16]. Superoxide dismutase, at concentrations as high as 1 mg/ml (2750 U/ml), did not prevent paraquat-induced inhibition of SAMDC (Table 1). Similarly, neither catalase nor the hydroxyl radical scavenger 1,3-dimethylurea afforded any protection to the enzyme. It was concluded from these studies that the inhibition of rat lung SAMDC by paraquat was not due to the generation of oxygen metabolites.

Figure 1 illustrates the effect of paraquat on SAMDC in the presence of a constant concentration of *S*-adenosylmethionine (3.3 μM). Reciprocal plot of the data indicated that paraquat induced a classically noncompetitive inhibition of the enzyme. Kinetic constants were determined by nonlinear least squares regression of the data assuming Michaelis–Menten kinetics. The calculated K_ms were 184 μM and 173 μM in the absence and presence of paraquat respectively. The maximum velocities for the reaction were 120 and 43 pmol/60 min/g lung respectively.

Since putrescine is an activator of SAMDC but is not a substrate for the enzyme, experiments were undertaken to examine whether paraquat inhibited SAMDC by altering the binding of substrate, *S*-adenosylmethionine. In these studies, enzyme activity was determined at a constant putrescine concentration (1 mM) and a range of *S*-adenosylmethionine concentrations in the presence and absence of paraquat (Fig. 2). A K_m of 68 μM and a maximum velocity of 1475 pmol/60 min/g lung was calculated in the absence of inhibitor. A K_m of approximately 90 μM has been reported for partially purified SAMDC from mouse liver [17]. In the presence of paraquat, *S*-adenosylmethionine-dependent SAMDC activity illustrated a distinctly nonlinear reciprocol plot. The significance of these data is more clearly illustrated in Fig. 3 where the data has been presented as an Eadie–Scatchard plot. Clearly, SAMDC kinetics in the presence of paraquat do not obey classical Michaelis–Menten kinetics. The mechanism underlying this effect is presently unknown. A possible explanation is that the binding of paraquat to SAMDC can induce conformation changes in the enzyme that alter the binding capabilities of both substrate and activator (putrescine). It should be emphasised that enzyme kinetics in a system as complex as 110,000 *g* supernatants may be complicated by the heterogeneous nature of the preparation. Studies using partially purified SAMDC are under way in order to elucidate further the mechanism of paraquat inhibition of the enzyme.

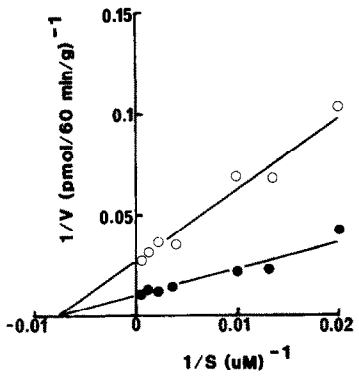

Fig. 1. Reciprocal plots of putrescine-stimulated SAMDC activity in rat lung in the presence (○) and absence (●) of 1 mM paraquat. Lung cytosol (0.5 ml) was incubated with 0.2 μCi *S*-adenosylmethionine, 1 mM pyridoxal-5-phosphate, 1 mM dithiothriotol and 0.1 mM EDTA in a final volume of 1 ml of 100 mM phosphate buffer. Generated $^{14}CO_2$ was collected and quantified as a measure of SAMDC activity. Each point is the mean of 3–5 observations. Standard errors ranged from 3 to 12% of the means.

Table 1. Effect of paraquat and 4,4′-dipyridyl on rat lung SAMDC

Treatment	SAMDC activity (pmol/60 min/g lung)
Control	62.5 ± 6.7
Paraquat (0.01 mM)	55.7 ± 4.3
Paraquat (0.1 mM)	40.7 ± 3.7*
Paraquat (1.0 mM)	13.2 ± 2.9*
4,4-Dipyridyl (0.1 mM)	64.9 ± 8.3
4,4-Dipyridyl (1.0 mM)	72.0 ± 5.7
Paraquat (1 mM) + catalase (10 μg/ml)	13.1 ± 1.3*
Paraquat (1 mM) + superoxide dismutase (1 mg/ml)	18.7 ± 2.7*
Paraquat (1 mM) + 1,3-dimethylurea (1 mM)	10.1 ± 3.1*

Data are presented as pmol of $^{14}CO_2$ produced/60 min/g lung (mean ± S.E.M., N = 5). Asterisk indicates values significantly different ($P < 0.05$) to control. Final concentration of putrescine and *S*-adenosylmethionine was 1 mM and 3.3 μM respectively.

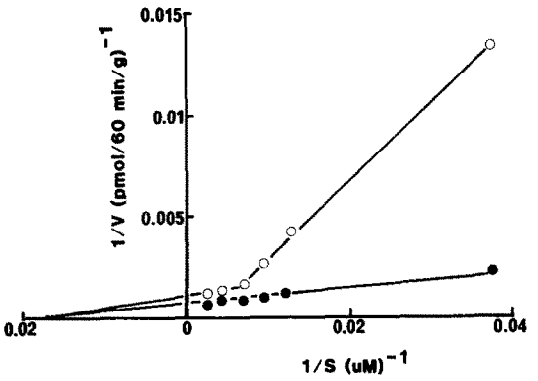

Fig. 2. Reciprocal plots of *S*-adenosylmethionine-dependent SAMDC activity in rat lung in the presence (○) and absence (●) of 1 mM paraquat. Each point is the mean of 3–6 observations. Standard errors ranged from 7 to 16% of the means. For experimental detail, see Fig. 1.

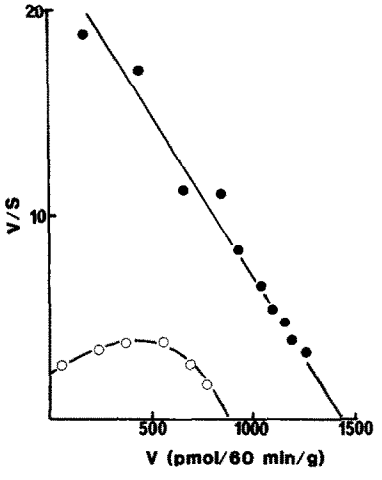

Fig. 3. Eadie–Scatchard plot of *S*-adenosylmethionine-dependent SAMDC activity in rat lung. A linear plot was obtained in the absence of paraquat (●). In the presence of 1 mM inhibitor (○), the Eadie–Scatchard plot demonstrated a concave curve with a positive slope over the substrate range of 25–150 μM.

Although structurally unsimilar to the dipyridyls, bis(guanylhydrazones) are potent inhibitors of SAMDC. The kinetics of MGBG inhibition of rat liver SAMDC have been reported and show that MGBG is a competitive inhibitor with *S*-adenosylmethionine but uncompetitive with putrescine [18]. Like paraquat, MGBG does not appear to compete directly for the putrescine binding site on the enzyme. However, based on the kinetics of each inhibitor with SAMDC, it would appear that paraquat and MGBG do not interact with the enzyme in similar manners.

In support of our original hypothesis, paraquat was found to inhibit rat lung SAMDC activity. However, the mechanisms of inhibition does not appear to be by simple competitive displacement of putrescine from its active site.

The postulated interaction of polyamines with macromolecules is relatively simple in nature involving ionic binding of the positively charged nitrogen moieties of the polyamines with negatively charged sites such as phosphate groups on double strand DNA or phospholipids [1, 10]. However, the interaction of polyamines with cellular macromolecules appears to be a regulatory mechanism for many cellular functions. Indeed, most cells require polyamines in order to proliferate [2]. This fact has been vigorously pursued as a possible mechanism for chemotherapeutic control of cancer cell growths. The ODC inhibitor DFMO has demonstrated antitumor activity in rodent models although it has shown less dramatic effects in patients [19]. One consequence of treating certain tumor cells with DMFO is an increased ability of those cells to accumulate extracellular polyamines [20]. The 4,4′-dipyridyl analogs may represent a new class of antipolyamine drugs with potential chemotherapeutic activity, especially if they are taken up into tumor cells in a manner similar to that of the polyamines.

Drug Metabolism and Toxicology Section,
Department of Pharmacology,
University of Western Australia,
Nedlands, W.A. 6009, Australia
RODNEY F. MINCHIN

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