REVERSIBLE INHIBITION OF RAT LIVER REGENERATION BY 1,3-DIAMINO-2-PROPANOL, AN INHIBITOR OF ORNITHINE DECARBOXYLASE

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1. Introduction

Rodent liver regeneration in response to tissue loss is characterized by an early and dramatic accumulation of putrescine [1] and spermidine [2,3] in the remaining liver remnant and results from an intense stimulation of the enzyme ornithine decarboxylase (EC 4.1.1.17) [4,5]. Several lines of experimental evidence suggest that the enhanced accumulation of polyamines is specifically required for a proper proliferative response of the liver tissue. Repeated injections of 1,3-diaminopropane, an indirect inhibitor of ornithine decarboxylase, not only prevented the prereplicative accumulation of putrescine and spermidine [6,7] but also produced a profound inhibition of the stimulation of liver DNA synthesis normally occurring after the partial resection of the liver [7,8]. The idea that the antiproliferative effect exerted by diaminopropane was mediated through a polyamine depletion was further supported by the findings in [9] demonstrating that a single post-operative injection of diaminopropane, which, as such, was without any effect on spermidine accumulation or DNA synthesis, combined with an irreversible inhibitor [1,1'-(methylethanediylidenedinitrilo)-bis-(3-aminoguanidine)] [10] of *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50), similarly ineffective alone, resulted in a delayed accumulation of spermidine and a complete inhibition of DNA synthesis in regenerating rat liver. The above-cited experiments, however, have been of relative short duration and there are practically no data available of the effect of polyamine depletion on the ultimate result of liver regeneration as defined by the actual restoration of the tissue mass over a period of several days.

We have found that a close analog of 1,3-diaminopropane, namely 1,3-diamino-2-propanol, is equally or more effective in depressing ornithine decarboxylase activity in vivo than the parent compound, and apparently possesses a longer duration of action. The latter compound can also be administered orally with no need for repeated injections. We will now show that the inclusion of diaminopropanol in the drinking water of partially hepatectomized rats resulted in a complete or near complete inhibition of ornithine decarboxylase activity and likewise prevented any accumulation of spermidine and spermine for a period of several days. Four days after partial hepatectomy, liver regeneration (weight gain) was virtually totally inhibited by the compound and apparently resulted from a gradually strengthening inhibition of DNA synthesis. Withdrawal of the drug after 2 days following the operation initiated the regeneration again as judged by a rapid increase in liver weight, enhanced accumulation of spermidine and increased synthesis of DNA.

2. Materials and methods

2.1. *Animals and treatments*

Female rats of the Wistar strain (weighing about 200 g) were used in all experiments. Partial hepatectomy was performed under light ether anaesthesia as in [11]. The animals were fed standard rodent chow, the drinking water being replaced during the actual experiments by a commercial lingonberry juice to mask the taste of diaminopropanol.

2.2. *Chemicals*

D,L-[1-^{14}C]Ornithine (59 mCi/mmol), [6-^{3}H]-thymidine (26.4 Ci/mmol) and [6-^{14}C]orotic acid (58 mCi/mmol) were purchased from the Radiochemical Centre (Amersham). *S*-Adenosyl-L-[1-^{14}C]-methionine was prepared enzymically as detailed in [12]. 1,3-Diamino-2-propanol was purchased from Fluka AG (Buchs, SG). The lingonberry juice was obtained from a local grocery.

2.3. *Analytical methods*

Ornithine decarboxylase [13], adenosylmethionine decarboxylase [14] and thymidine kinase (EC 2.7.1.2) [15] activities were measured by the published methods. The synthesis of DNA and RNA was measured by injecting the rats with 10 μCi of [^{3}H]thymidine together with 2.5 μCi [^{14}C]orotic acid 30 min before death. RNA was measured after alkaline digestion as in [16] and DNA after acid hydrolysis as in [17]. Spermidine and spermine were measured as in [18] and protein as in [19].

The significance of the differences was estimated using two-sided Student's *t*-test.

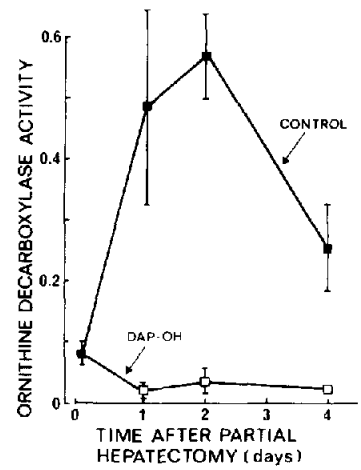

Fig.1. Inhibition of ornithine decarboxylase activity by 1,3-diamino-2-propanol in regenerating rat liver. After partial hepatectomy the animals received a single intraperitoneal injection of diaminopropanol (100 μmol/100 g body wt) or no treatment. The drinking water of the rats was replaced by berry juice without (control) or with 100 mM diaminopropanol (DAP-OH). There were 2 (no error bars) to 4 animals in each group. The vertical bars represent standard error of the mean (SEM).

3. Results

A single injection of 1,3-diamino-2-propanol (100 μmol/100 g body wt) at the time of partial hepatectomy and the subsequent addition of 100 mM diaminopropanol into the drinking water of the rats abolished any stimulation of ornithine decarboxylase activity in the regenerating liver over a period of 4 days (fig.1). The consumption of the drug (berry juice-flavored drinking water) was surprisingly constant as indicated by the small variations of ornithine decarboxylase activities between individual rats receiving the drug (fig.1). As shown in table 1, some-

Table 1
Effect of 1,3-diamino-2-propanol on rat liver regeneration

Treatment	Liver wt (g)	Spermidine (μmol/liver)	Spermine (μmol/liver)	DNA (mg/liver)	DNA (mg/liver)
Unoperated controls (4)	2.98 ± 0.07[a]	2.94 ± 0.24[a]	2.16 ± 0.24[a]	4.55 ± 0.06[a]	18.6 ± 0.50[a]
Partial hepatectomy (8)	5.00 ± 0.12	6.24 ± 0.23	2.66 ± 0.13	9.51 ± 0.36	37.2 ± 1.07
Partial hepatectomy + diaminopropanol (7)	2.92 ± 0.07[b]	2.46 ± 0.12[b]	2.00 ± 0.07[b]	6.17 ± 0.17[b]	19.8 ± 0.45[b]

[a] Refers to the lobes remaining after partial hepatectomy
[b] $p < 0.001$; the significance of the differences produced by diaminopropanol

After partial hepatectomy the animals received a single intraperitoneal injection of diaminopropanol (100 μmol/100 g body wt) or no treatment. The drinking water of all rats (including unoperated controls) was replaced by berry juice, without or with 75 mM diaminopropanol. The results are given ± SEM. The number of animals in each group is given in parentheses

what lower concentration (75 mM) of diaminopropanol totally prevented the almost 2-fold increase in the weight of the remaining lobes normally found after 4 days following partial hepatectomy. Nor was there any net accumulation of spermidine or spermine in livers of rats receiving the drug. Almost total was likewise the prevention of liver DNA and RNA accumulation in diaminopropanol-treated animals (table 1).

Figure 2 presents a closer analysis of the effects produced by diaminopropanol during the course of rat liver regeneration. Although there appeared to be a small weight increase (possibly partly due to the water retention) during the first 2 days after partial hepatectomy also in rats on diaminopropanol, liver

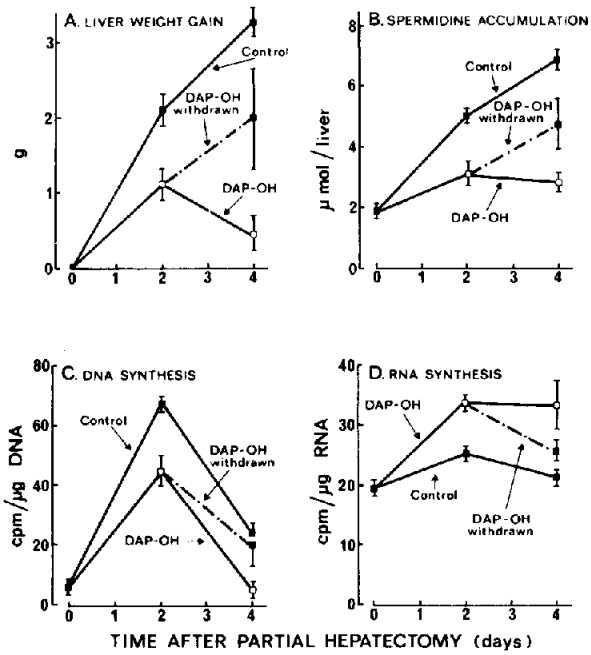

Fig.2. Effect of 1,3-diamino-2-propanol on liver weight gain, spermidine accumulation, DNA synthesis and RNA synthesis in regenerating rat liver. After partial hepatectomy the animals received a single intraperitoneal injection of diaminopropanol (100 μmol/100 g body wt) or no treatment. The drinking water of the rats was replaced by berry juice without (control) or with 75 mM diaminopropanol (DAP-OH). Diaminopropanol was withdrawn after 2 days from one group of rats (DAP-OH withdrawn) and the animals continued with the berry juice alone for the next 2 days. There were 4–5 animals in each group. The vertical bars represent standard error of the mean (SEM).

regeneration definitely stopped between 2 and 4 days post-operatively (fig.2A). The withdrawal of the drug after 2 days seemed to initiate the regeneration process again as seen in the rapid weight gain of the liver (fig.2A). Spermidine accumulation was prevented almost totally, but not irreversibly, by diaminopropanol(fig.2B). The incorporation of labelled thymidine into liver DNA was inhibited by about 35% ($p < 0.01$) at 2 days and by 75% ($p < 0.001$) at 4 days following partial hepatectomy in diaminopropanol-treated rats (fig.2C). Slightly surprising was the finding that even though the net accumulation of liver RNA was profoundly inhibited (table 1), the incorporation of orotic acid into total liver RNA appeared to proceed even at higher rate in animals receiving diaminopropanol in their drinking water (fig.2D). The idea that the enhancement of the synthesis of RNA was produced by diaminopropanol was supported by the finding that the withdrawal of the drug rapidly 'normalized' the incorporation pattern (fig.2D).

During the first 2 days, diaminopropanol effectively prevented the rise in liver spermidine concentration apparently due to the markedly lowered activity of ornithine decarboxylase (table 2). As shown also in table 2, the activity of adenosylmethionine decarboxylase and that of thymidine kinase were not inhibited to any appreciable extent at this time point. This finding appears to strengthen the view that the inhibition of ornithine decarboxylase activity as well as the depression of thymidine incorporation into liver DNA (fig.2C) was not due to any general toxic effects since adenosylmethionine decarboxylase [20] and thymidine kinase [21] are enzymes known to possess a rapid turnover rate, just like ornithine decarboxylase [22], thus being sensitive markers of any disturbances of the synthesis of liver proteins.

Four days after the surgery, diaminopropanol still suppressed the increase in spermidine accumulation and ornithine decarboxylase activity while the activity of adenosylmethionine decarboxylase was unaffected (table 2). The decrease in the specific activity of thymidine kinase (about 50%) brought about by diaminopropanol in 4 days (table 2) was substantially less than the inhibition seen in the synthesis of DNA in vivo (fig.2C).

In every instance, the changes produced by diaminopropanol were at least partly reversed by the withdrawal of the drug (table 2).

Table 2
Effect of 1,3-diamino-2-propanol on polyamine synthesis and accumulation and on the activity of thymidine kinase

Treatment	Days after part. hepatec.	Spermidine (μmol/g wet wt ± SEM)	Spermine (μmol/g wet wt ± SEM)	Ornithine decarboxylase act.	Adenosylmethionine decarboxylase act.	Thymidine kinase act.
Unoperated controls (5)	0	0.67 ± 0.03	0.58 ± 0.01	0.05 ± 0.01	0.26 ± 0.04	0.24 ± 0.02
Partial hepatectomy (5)	2	1.02 ± 0.03	0.37 ± 0.01	0.40 ± 0.08	0.49 ± 0.04	3.56 ± 0.49
Partial hepatectomy + diaminopropanol (5)	2	0.77 ± 0.04[c]	0.40 ± 0.01	0.13 ± 0.03[a]	0.65 ± 0.06	3.49 ± 0.51
Partial hepatectomy (5)	4	1.13 ± 0.05	0.44 ± 0.02	0.11 ± 0.01	0.22 ± 0.03	0.61 ± 0.04
Partial hepatectomy + diaminopropanol (5)	4	0.87 ± 0.07[a]	0.58 ± 0.03[b]	0.06 ± 0.01[b]	0.19 ± 0.05	0.32 ± 0.03[c]
Partial hepatectomy + diaminopropanol (withdrawn after 2 days) (4)	4	0.96 ± 0.06	0.49 ± 0.02	0.18 ± 0.08	0.20 ± 0.04	0.51 ± 0.12

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$

The significance of the differences produced by diaminopropanol. The enzyme activities are given in nmol/mg protein ± SEM. For experimental details see the legend for fig.2. The number of animals in each group is given in parentheses

4. Discussion

The prevention of polyamine accumulation by competitive inhibitors of ornithine decarboxylase [23–25] or inhibitors of adenosylmethionine decarboxylase [26–29] under a variety of experimental conditions involving accelerated growth appears to result in profound disturbances in cell proliferation, especially in DNA synthesis. Indirect amine inhibitors of ornithine decarboxylase (such as 1,3-diaminopropane), which may act through an induction of protein inhibitors to the enzyme [30] or through more direct transcriptional or translational control mechanisms [31], have likewise been employed to abolish polyamine accumulation in regenerating rat liver [7–9], in rat liver during refeeding [32] and ovary cells grown in culture [33]. In every instance, the prevention of enhanced spermidine and/or putrescine accumulation was associated with distinct decreases in the synthesis of DNA. However, due to the rapid metabolism, the inhibition of ornithine decarboxylase in vivo by compounds like diaminopropane required multiple injections [6–8, 32] which, in addition to the obvious experimental inconveniences, may give rise to toxic side-effects owing to high, albeit transient, tissue concentrations of the compound. It thus appeared to us that an inclusion of diaminopropanol in the drinking water of the animals would offer a more gentle way for the administration of the inhibitor. As shown in the present results, peroral diaminopropanol produced a virtually complete prevention of rat liver regeneration as judged by the ultimate variable of the regeneration process, namely liver weight gain. In aggreement with [7–9,27,28], the synthesis of DNA seemed to be affected mostly while the synthesis of RNA apparently proceeded as in the absence of the inhibitors or, according to the present results, even at an enhanced rate.

Even though the present results definitely show that the inhibition of polyamine synthesis in whole animals is associated with a prevention of liver regeneration, it is exceedingly difficult to exclude all secondary effects, which possibly contribute to the antiproliferative action, exerted by a compound like diaminopropanol. This may even remain unsolved since the toxicity of higher polyamines (spermidine and spermine) makes it very difficult to perform any straightforward reversion experiments in whole animals.

There exist, however, some experimental findings suggesting that the effect of diaminopropanol would not be based on general toxicity:

(i) The synthesis of RNA was not depressed by the drug.

(ii) The activity of adenosylmethionine decarboxylase and that of thymidine kinase, both extremely sensitive indicators of unimpaired protein synthesis (owing to their rapid turnover rate), were not initially affected.

(iii) The general conditions of rats received diaminopropanol was rather good even though they lost more (about 20%) weight than partially hepatectomized rats in general. However, in the absence of liver regeneration for several days one should already expect some signs of liver insufficiency.

The molecular mechanisms through wich diaminopropanol or propanol-induced polyamine depletion brings about the antiproliferative action remains to be determined. It is thus unlikely that the inhibition of DNA synthesis resulted from an inhibition of the induction of thymidine kinase, as recently proposed as one of the antiproliferative actions of methylglyoxal bis-(guanylhydrazone) in lymphocytes [34], since the marked enhancement of the activity of this enzyme occurred as in the absence of the drug (table 2) and as described for regenerating rat liver [35].

A puzzling observation was likewise the virtually total block of RNA accumulation by diaminopropanol (table 1) while the incorporation of radioactive orotate appeared to continue undisturbed, at least at the time points measured (fig.2D). This could be understood in terms of an enhanced degradation of RNA in the absence of sufficient levels of polyamines.

An important piece of additional information is also included in the present results: regardless of the mechanism of action, the inhibition of liver regeneration by diaminopropanol was reversible as indicated by the reinitation of the regenerative process upon withdrawal of the drug.

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