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The toxic effects of periodate-oxidized adenosine given in divided doses to rats

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Adenosine dialdehyde, a periodate-oxidized adenosine (PAD or Adox), is a potent inhibitor of *S*-adenosylhomocysteine hydrolase (AdoHcy hydrolase; EC 3.3.1.1.) and through the accumulation of AdoHcy inhibits *S*-adenosylmethionine (AdoMet)-dependent transmethylation resulting in the inhibition of a number of physiological processes [1] and the methylation of arsenite [2] and selenite [3]. The effect of PAD on selenite metabolism is reflected by decreased dimethylselenide exhalation [3] and decreased urinary trimethylselenonium excretion [4]. The toxicity of this inhibitor is species-dependent: while mice can tolerate 100 μmol/kg without any ill-effect [2, 5], 50 μmol/kg killed, or made rats moribund, within 24 hr, while 15 μmol/kg was well-tolerated [3]. Studies presented here revealed that PAD given in 2 × 5 μmol/kg doses 6 hr apart was not only more toxic than a single dose of 10 or 15 μmol/kg, but produced novel toxic effects.

Materials and methods

Female Porton Wistar rats, 180–210 g body weight were used. Periodate-oxidized-adenosine (PAD) was synthesized and the solution prepared by injection according to Hoffman [5]. Rats were injected i.p. with either 5.0 ml saline/kg or 5.0, 2 × 5.0, 10.0 or 15 μmoles PAD/5 ml saline/kg body weight. Some of the animals 15 min after the single or the first of two injections of PAD were injected s.c. with a fresly-prepared solution of 12.0 μmoles/kg Na_2SeO_3 (BDH) in 2.5 ml/kg saline, which was labelled with ^{75}Se-selenite (Amersham International, Bucks, U.K.) to give approx. 5 μCi/ml. Other animals received only 2.5 ml saline instead of selenite. Immediately after injection some selenite-treated rats were placed in pairs in glass metabolic cages (Metabowls, Jencons, Leighton Buzzard, U.K.) with free access to water and food and the excretion of dimethylselenide was estimated from the presence of ^{75}Se in exhaled air. Air sucked through the cages at a rate of 2.5 l/min was passed through 4 translucent vinyl tubes (8 mm i.d.) each containing 1 g granular charcoal (8–20 Mesh) to absorb exhaled ^{75}Se. Absorbers were changed at 6 hr, when the 2 × 5 μmoles PAD-dose groups received the second dose. Twenty-four hours after the injection of selenite or saline the number of dead animals was noted.

In some experiments surviving rats were decapitated. Blood was collected from the severed vessels into heparinized beakers. Kidneys and livers were removed and fixed in buffered formalin for 14 days and then embedded in paraffin wax. Sections of 4 μm were cut and stained with haematoxylin and eosin. Selected liver sections were stained by the Fouchet method for bile plugs. The histological examination was carried out without reference to treatment.

Haematocrit was estimated and haemoglobin determined by means of a Haemoglobinometer (Coulter Electronics, Harpenden, Herts.). Plasma bilirubin concentration was estimated with the Peridochrom Bilirubin kit of Boehringer (Mannheim GmbH, F.D.R.). Absorbers were assayed for ^{75}Se by gamma-counting in a well-shaped sodiumiodide crystal of 8 cm dia. and 10 cm depth with an efficiency of 85%.

Differences between the mortality of groups were calculated with the Chi-square test (corrected for continuity) for independent samples or with the Fisher exact probability test when frequency was zero in one compartment [6]. Two-directional test was used to calculate statistical significance for total mortality, and one-directional test for the evaluation of selenite effect on mortality. The power of the Chi-square test was calculated according to Cohen [7]. Other data were first evaluated with the Kruskal–Wallis test of one-way analysis of variance. When this test rejected null hypothesis, significant differences between control and other groups (Table 2) or the 2 × 5 μmol/kg PAD group and other groups (Table 3) were calculated with the one-directional Mann–Whitney U-test [8].

Results

Table 1 shows that the administration of two doses of 5.0 μmol/kg PAD given 6 hr apart increased 24 hr mortality significantly compared with treatment with a single dose of 5, 10 or 15 μmol/kg. The administration of 12 μmol/kg Na_2SeO_3 15 min after a single dose of 15 μmol/kg PAD or after the first of two doses of 5 μmol/kg PAD increased mortality, but the increase did not reach the level of significance. However, the power of the one-directional Chi-square test for groups treated with 2 × 5 μmol/kg PAD was

Table 1. Twenty-four hr mortality of female rats given PAD in different doses with or without the administration of a single dose of 12 μmol/kg Na_2SeO_3. There was a 6 hr interval between the administration of two 5 μmoles/kg PAD and selenite was given 15 min after the first dose or a single dose of PAD

Dose of PAD (μmol/kg)	No.	Total mortality (%)	Without selenite mortality N	(%)	With selenite mortality N	(%)
5	69	0	57	0	12	0
2 × 5	64	35.9*	20	20	44	43.2†
10	63	0	12	0	51	0
15	34	5.9	4	0	30	6.6†

* Treatment with 2 × 5 μmol/kg PAD caused significantly more mortality ($P < 0.05$) with the two-directional Chi-square test than any other treatment.

† Selenite treatment did not increase mortality significantly (one-directional Chi-square test) in rats given 2 × 5 μmol/kg (power = 0.56) or 15 μmol/kg (PAD) (Fisher exact probability test).

low (0.56) and therefore the probability of Type II error was high. Actually with the same effect size, but with N = 77 instead of 64, the difference would be significant.

Autopsy of the 5, 10 or 15 μmol/kg PAD groups revealed no abnormalities with the exception of mottled liver in the two higher dose groups. After two doses of 5 μmol/kg PAD the livers were mottled, enlarged, congested and bile duct dilated. Pancreas showed a yellowish bilious discolouration. Gastric haemorrhage, usually 2–3 mm dia. in the glandular epithelium also was frequent and occasionally reached the external surface.

Histology. No hepatic or renal damage was observed in rats treated with selenite alone, but PAD-treated rats, especially when the total dose was 10 μg/kg or more had well-defined damage in these two organs. There were eight animals in each PAD-dose group and half of them received selenite.

5 μmol/kg PAD: Without selenite only one animal, and with selenite treatment three rats had mild mid-zonal hydropic degeneration. Only one rat (selenite-treated) had mild inflammatory cell infiltration in the renal cortex.

10 μmol/kg PAD: in the non-selenite group one animal had mild hydropic degeneration and necrosis in the mid-zonal region of liver, and one had Kupffer cell necrosis. Kupffer cell necrosis was seen in one of the selenite-treated rats and mild mid-zonal hydropic degeneration in all. Half of the rats without selenite and all four rats treated with selenite developed some cellular infiltration in the renal cotex.

15 μmol/kg PAD: Mid-zonal hydropic degeneration was a common sign. In the non-selenite group, in addition to hydropic degeneration, one animal also had necrosis and teleangiectasia, and in the selenite group one had periportal necrosis and another Kupffer cell necrosis. Three of the rats without selenite treatment developed tubular necrosis; one in the straight part with cellular infiltration distally, one in the proximal part of the convolutions, and one in each part of the proximal tubules. Three of the selenite group had cellular infiltration mainly in the proximal convolutions, and two of these also had moderate necrosis in the straight part.

2 × 5 μmol/kg PAD: Severe hepatic congestion with hydropic degeneration, mid-zonal and Kupffer cell necrosis were common hepatic signs. In the selenite group two animals also had cavernous dilation of sinusoids. All animals given 2 × 5 μmol/kg PAD developed renal proximal tubular necrosis, which was extensive in the straight part.

Table 2 shows that only 2 × 5 μmol PAD decreased haematocrit or haemoglobin concentration and increased plasma bilirubin concentration. The plasma bilirubin concentration was higher in selenite-treated rats, than in rats without selenite, but the difference was not significant. Table 3 shows that using the exhalation of ^{75}Se as a measure of selenium dimethylation, 2 × 5 μmol/kg PAD inhibited

Table 2. Effects of PAD with or without 12 μmol/kg Na_2SeO_3 on haematocrit, haemoglobin and plasma bilirubin. Animals were killed 24 hr after the administration of PAD. Treatment schedule was the same as in Table 1. (The numbers in brackets give the number of animals)

Dose of PAD (μmol/kg)	Selenite	Haematocrit (%)	Mean ± SEM haematoglobin (g%)	Bilirubin (μmol/l plasma)
—	—	43.0 ± 0.63 (8)	13.6 ± 0.25 (8)	1.9 ± 1.37 (4)
5	—	44.2 ± 1.38 (4)	13.8 ± 1.01 (4)	2.39 ± 0.98 (4)
5	+	44.3 ± 0.86 (7)	15.0 ± 0.32 (4)	2.27 ± 0.56 (12)
2 × 5	–	36.8 ± 1.07* (10)	12.2 ± 0.45* (15)	39.7 ± 3.23* (13)
2 × 5	+	34.1 ± 2.11* (5)	11.5 ± 0.64* (5)	50.8 ± 6.42* (5)
10	–	41.7 ± 0.25 (4)	12.9 ± 1.00 (4)	2.0 ± 1.43 (4)
10	+	46.5 ± 0.64 (4)	14.2 ± 0.61 (4)	3.11 ± 0.72 (5)

* Significantly decreased haematocrit or haemoglobin concentration and significantly increased plasma bilirubin level with the one directional Mann–Whitney U-test ($P < 0.05$).

Table 3. Effects of PAD given in different doses on the exhalation of ^{75}Se in 0–6, 6–24 and 0–24 hr periods after the administration of 12 μmoles/kg $Na_2\ ^{75}SeO_3$. For treatment schedule see Table 1

Dose of PAD (μmoles/kg)	N	Exhalation of ^{75}Se in % of dose (mean ± SEM) 0–6 hr	6–24 hr	0–24 hr
0	10	13.9 ± 1.32*	5.7 ± 0.41*	19.6 ± 1.76*
5	5	0.97 ± 0.10	4.9 ± 0.56*	5.8 ± 0.47*
2 × 5	5	0.69 ± 0.17	0.42 ± 0.03	1.11 ± 0.20
10	4	0.28 ± 0.04	1.40 ± 0.50*	2.56 ± 0.39*
15	4	0.21 ± 0.06	1.17 ± 0.63*	1.38 ± 0.32

* With the Mann–Whitney one-directional U-test these groups exhaled significantly more ($P < 0.05$) ^{75}Se than the group dosed with 2 × 5 μmol/kg PAD.

methylation more in the 6–24 hr period than single doses of 5.0, 10.0 or 15 μmol/kg PAD. In the whole 24 hr period rats treated with 2 × 5 μmol/kg PAD had the lowest level of respiratory loss of ^{75}Se, but the difference between the 2 × 5 and 15.0 μmol/kg groups was not significant.

Discussion

Inhibitors of AdoHcy hydrolase through the accumulation of AdoHcy inhibit AdoMet-dependent methylases by a feedback mechanism [9]. Research has been mainly focused on the antiviral and oncostatic properties of these inhibitors [9], but the present study indicates that adenosine dialdehyde, a periodate oxidized adenosine (PAD) exerts well-defined toxic effects in rats. As several low molecular weight compounds, like catechols, norepinephrine, histamine, serotonin and tryptamine, and also macromolecules, like proteins, nucleic acids and membrane phospholipid are substrates for AdoMet-dependent transmethylation [1, 9], the inhibition of transmethylation may affect an arsenal of physiological processes and may produce morphological lesions. Thus haemorrhage in the glandular part of the stomach may have been the consequence of the effect of PAD on the methylation of membrane phospholipid and histamine. One of the characteristics of PAD toxicity is that 5 μmol/kg dose given twice 6 hr apart, caused not only more severe or more widespread hepatic and renal proximal tubular damage than 10 or 15 μmol/kg did, but produced novel effects not seen in single-dosed animals.

Anaemia, haemorrhage in the glandular part of the stomach, enlarged congested liver, bilirubinaemia with signs of jaundice and significantly increased mortality were seen only in rats treated with 2 × 5.0 μmol/kg PAD. Mortality was further increased by selenite. Though the increase was not statistically significant, the low power of the test (see Result section) does not exclude the possibility that selenite increased the lethal toxicity of PAD. This view is supported by the findings of Hoffman and McConnel [4] who have found that mortality occurred only in mice treated with both 4 mg/kg selenite and 100 μmol/kg PAD but not in mice given only one of the two compounds [4].

Considering that the blood volume in rats of 200 g body weight is about 13 ml [10], rats treated with selenite and 2 × 5 μmol/kg PAD had 50.8 × 13 × 0.659 = 0.43 μmol plasma bilirubin. As the decrease in haemoglobin concentration from 13.6 g% to 11.5 g% corresponds to the loss of 17 μmol haemoglobin subunits and the transport maximum for bilirubin excretion is 14 μmol/hr in rats of the same body weight [11], haemolysis was unlikely to be the sole cause of PAD-induced bilirubinaemia. The presence of hydropic degeneration, congestion, periportal damage, cavernous sinusoids and dilated bile duct seen in livers suggests that a defect in the hepatic handling of bilirubin was also a contributory factor.

Comparison of ^{75}Se exhalation between the different dose regimes demonstrates that the group with the lowest degree of dimethylselenide exhalation (and therefore synthesis) had also bilirubinaemia and other signs of increased toxicity. As the inactivation of AdoHcy hydrolase by PAD shows saturability, and the unimolecular reaction between PAD and the enzyme is reversible [9], it seems reasonable to suggest that the total dose is less important in PAD-induced toxicity than the suppression of methylase activity below a threshold level for a prolonged period of time. While 2 × 5 μmol/kg PAD caused a 93% decrease in the exhalation of ^{75}Se, rats given 10 or 15 μmol/kg, PAD had 75 and 79% decrease respectively in the 6–24 hr period.

In summary, 20% of rats given only 10 μmol/kg PAD in two divided doses, but not in a single dose, died within 24 hr and the remaining 80% had loss of haemoglobin, bilirubinaemia, bile duct dilation, severe hepatic congestion and gastric haemorrhage. Selenite, though not statistically significantly, seemed to increase the effects of PAD on lethality, loss of haemoglobin and bilirubinaemia.

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Dichloro-*p*-nitroanisole (DPNA)-demethylation not specifically induced by phenobarbitone in mice

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DPNA was introduced as a substrate of the microsomal monooxygenase system of rats by Hultmark *et al.* [1, 2]. Its metabolism was found to be enhanced (calculated per mg microsomal protein) 24-fold after phenobarbitone pretreatment, but only very little after pretreatment with 3-methylcholanthrene. Thus, it seemed to be a substrate of considerable inducer-specificity. When looking for such substrates we investigated DPNA-demethylation after pre-