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STUDIES ON POISONING BY *MACROZAMIA COMMUNIS*—II

EFFECTS OF MODIFYING AGENTS UPON CHANGES INDUCED IN THE LIVER BY *MACROZAMIA COMMUNIS*

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Abstract—The periacinar necrosis and accumulation of triglyceride in the livers of rats produced by oral administration of the kernels of *Macrozamia communis* was prevented by promethazine hydrochloride and by adenosine. Treatment with promethazine and adenosine produced a marked hypothermia. Protection against macrozamia poisoning by promethazine was dependent upon body temperature. Adenosine protected against some of the early manifestations of macrozamia poisoning in rats whose body temperatures were maintained slightly above normal. Thiol and anti-oxidant pretreatment failed to protect against macrozamia poisoning. The results are discussed in relation to the possible mode of action of the toxic constituent of the kernels.

ORAL administration to rats of the kernels of *Macrozamia communis* has been shown to induce periacinar necrosis, accumulation of triglyceride, changes in lyosomal membranes and inhibition of protein synthesis.[1]

Promethazine hydrochloride protects against the hepatotoxic effects of thioacetamide[2] and ameliorates some of the manifestations of poisoning by carbon tetrachloride and dimethylnitrosamine (DMN).[3,4] Adenosine triphosphate and some of its precursors prevent the development of fatty livers in rats treated with ethionine.[5]

Cysteine and cystaeamine have been shown to prevent the toxic effects of some nitrosamines.[6,7] Macrozamine, or rather its aglycone, methylazoxymethanol, and the nitrosamines may have a common mode of action, one of alkylation.[8] Antioxidants such as *N*, *N*-diphenyl-*p*-phenylene diamine (DPPD) have been shown to exert some protective effect upon poisoning by CCl_4.[9]

The protective effects of certain procedures and agents against the hepatotoxic effects of CCl_4 have been shown to be dependent upon the induction of a state of hypothermia.[10,11]

Hence the effects of treatment with promethazine, adenosine, thiol agents and DPPD in preventing the toxic effects of oral administration of the kernels of *M. communis* were investigated. The effect upon body temperature of agents that protect against macrozamia poisoning was studied.

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EXPERIMENTAL

Animals and materials used were as described in the preceding paper.[1] Determination of enzyme activities and other methods were as previously described. An aqueous extract of the kernels of *M. communis* was administered to rats as an oral dose equivalent to 1·5 g of fresh kernel per 100 g body weight.

Promethazine hydrochloride was given as an i.p. injection at a dose of 25 mg/kg at 0 hr and then 12·5 mg/kg at 4 and 8 hr. ATP and precursors were administered subcutaneously at doses of 100 mg at 0, 2, 5 and 8 hr. Cysteine, cystaeamine and DPPD were given to rats as described by previous investigators.[6,9,7]

Groups of 4 to 6 rats were dosed with the extract of the kernels and some were treated with the protective agents. Other groups of rats were treated with the protective agents only. Rats were destroyed at intervals after poisoning and liver tissue was taken for histological and biochemical examination.

Rectal temperatures were measured by a thermo-couple inserted 1 cm into the rectum.

RESULTS

(i) Effects of protective agents upon histological changes caused by macrozamia

Promethazine, ATP, AMP and adenosine protected against the rapid fading of cytoplasmic basophilia, lipid accumulation and periacinar necrosis produced by macrozamia. On the other hand, neither cysteine, cystaeamine nor DPPD pretreatment resulted in any alteration in the histological changes caused by macrozamia.

(ii) Effects of promethazine and adenosine upon biochemical changes in the liver produced by macrozamia administration

Both promethazine and adenosine prevented the inhibition of NAD-dependent enzymes of mitochondria apparent 6 hr after macrozamia poisoning (Table 1).

TABLE 1. OXIDATION OF SUBSTRATES BY MITOCHONDRIAL PREPARATIONS

Treatment	L-Glutamate	L-Malate	Succinate
Control	104 ± 4	86 ± 11	247 ± 24
Adenosine	101 ± 12	73 ± 6	198 ± 8
Macrozamia	65 ± 8	58 ± 4	196 ± 13
Adenosine and macrozamia	96 ± 8	85 ± 4	226 ± 13
Control	119 ± 9	79 ± 7	222 ± 16
Promethazine	123 ± 9	79 ± 7	238 ± 15
Macrozamia	80 ± 10	64 ± 7	239 ± 23
Promethazine and macrozamia	115 ± 5	77 ± 4	220 ± 12

System: as in previous paper.[1] Animals poisoned for 12 hr. Results are expressed as μl O_2 taken up/mg mitochondrial N/20 min ± S.E.M.

Adenosine treatment caused a slight decrease in lipid levels in the livers of control rats. Promethazine and adenosine effectively protected against the accumulation of lipid in the liver of poisoned rats (Table 2).

Treatment of poisoned rats with promethazine or adenosine prevented the inhibition of incorporation of ^{14}C leucine into proteins of post-mitochondrial fractions apparent 6 hr after administration of macrozamia. (Table 2). Treatment of control rats with either agent doubled the incorporation of leucine into proteins of post-mitochondrial fractions isolated from these rats. Either agent prevented the inhibitory effect

TABLE 2. EFFECTS OF MODIFYING AGENTS UPON BIOCHEMICAL CHANGES PRODUCED IN THE LIVER BY *M. communis* KERNELS

Treatment	Lipid content mg lipid/g liver wet weight	Leucine incorporation into proteins of P.M. fractions % of control	Leucine incorporation into liver proteins *in vivo* % of control	Serum β-glucuronedase activity % of control	Hepatic ATP content μmoles ATP/100 mg weight
Control	40	—	—	—	0·95
Adenosine	36	243	120	50	0·63
Promethazine	38	223	134	80	0·54
Macrozamia	50	41	29	560	0·65
Adenosine and Macrozamia	33	228	93	92	0·57
Promethazine and Macrozamia	39	207	137	140	0·63
ATP	—	—	—	—	1·06
ATP and Macrozamia	—	—	—	—	0·95

Method: As in previous paper.[1] *In vivo* leucine incorporation studies two rats were each given 0·75 μc of ^{14}C-l-leucine i.p. 1 hr before destruction. Results are the means of four rats except where stated above. Rats destroyed 6 hr after poisoning except in ATP studies, 12 hr after poisoning.

of macrozamia upon *in vivo* incorporation of ^{14}C-leucine into liver proteins (Table 2).

Hepatic ATP levels in rats destroyed 12 hr after treatment with either promethazine or adenosine were lower than those in control rats. ATP levels were depressed in the livers of macrozamia-poisoned rats treated with these agents. On the other hand ATP administration maintained normal hepatic ATP levels after poisoning (Table 2).

The rise in serum β-glucuronidase activity, observed 6 hr after dosing with macrozamia, was prevented by the administration of promethazine and adenosine (Table 2).

The increase in the rate of release of β-glycerophosphate phosphatase from light mitochondrial (LM) fractions, observed 6 hr after poisoning with macrozamia, was prevented by adenosine treatment. However promethazine failed to do so. In fact, it stimulated an increase in the rate of release of the enzyme from LM fractions isolated from control rats (Fig. 1).

(iii) Effect of promethazine and adenosine upon body temperature

Rats treated with promethazine and adenosine experienced a marked hypothermia. However, rats kept in a hot room at 37° maintained their body temperature at slightly elevated levels after treatment with these agents (Fig. 2). Rats kept in the hot room and treated with adenosine rarely survived more than 6 hr.

(iv) Effect of promethazine and adenosine upon histological changes induced by macrozamia in the livers of rats in the hot room.

Adenosine-treated rats were destroyed 4 hr and promethazine-treated rats at 6 hr and 12 hr after treatment. Fading of cytoplasmic basophilia and accumulation of lipid in the centriacinar cells apparent 4 hr after macrozamia poisoning was prevented by adenosine. However, promethazine afforded no protection against the histological changes observed 6 and 12 hr after poisoning.

(v) Effect of agents upon biochemical changes produced by macrozamia in the livers of rats kept in the hot room

Adenosine but not promethazine prevented the accumulation of lipid in the livers

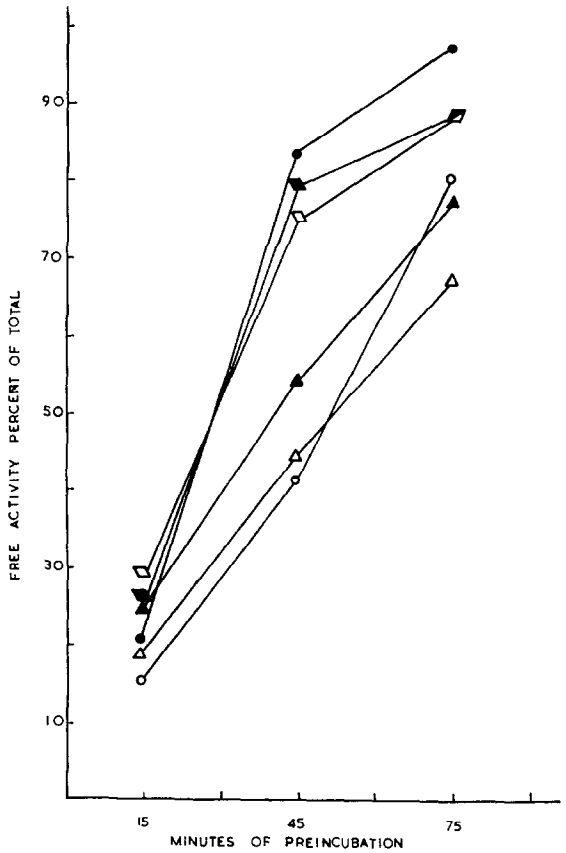

FIG. 1. Activation of β-glycerophosphate phosphatase in light mitochondrial fractions. Control ○—○; macrozamia ●—●; promethazine □—□; promethazine and macrozamia ■—■; adenosine △—△; adenosine and macrozamia ▲—▲. System: As previously described;[1] animals destroyed 6 hr after poisoning. Results are the means of two experiments all determinations performed in duplicate.

of rats kept in the hot room after macrozamia poisoning (Table 3). Adenosine also presented the increase in the rates of release of β-glycerophosphate phosphatase from LM fractions isolated from the livers of rats destroyed 4 hr after macrozamia poisoning (Table 3).

Promethazine at 0 and 4 hr failed to prevent the inhibition of mitochondrial glutamic dehydrogenase produced by macrozamia at 6 hours. However, if the second dose was delayed an hour the protective effect of promethazine was evident.

DISCUSSION

The possible mechanisms of action of agents modifying the effects of hepatoxic substances have recently been reviewed.[12,13] Attention has been drawn to the non-specific protection against the effects of CCl_4 or $CHCl_3$ poisoning afforded by subcutaneous or intraperitoneal injections of irritant or insoluble substances. This type of protection occurs when the substances are administered prior to the hepatotoxic agent.[14] Adenosine and promethazine prevent the expression of some manifestations

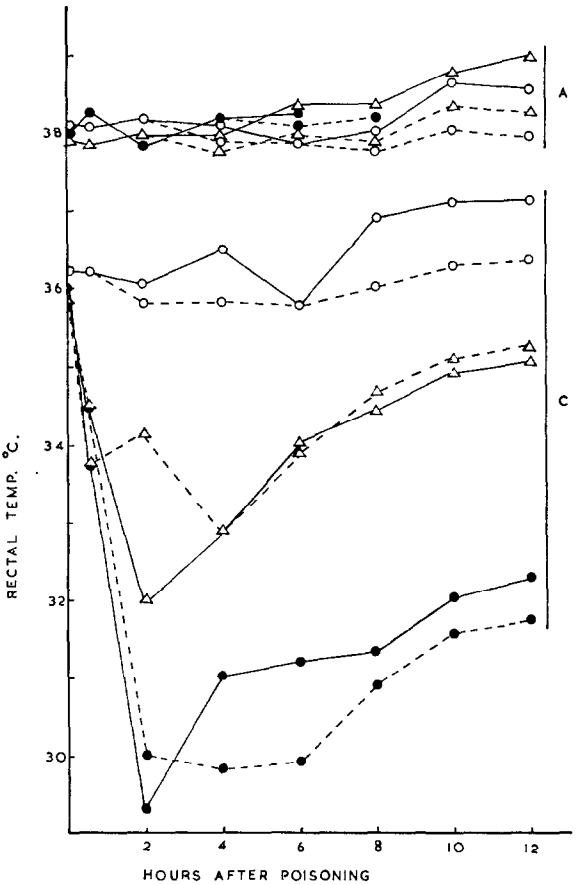

FIG. 2. Rectal temperatures of rats kept C—at ambient temperatures A—in room at 37°. Intact lines —control; broken lines—macrozamia poisoned; untreated ○—○; adenosine ●—●; promethazine △—△.

of macrozamia poisoning when administered simultaneously with and subsequently to the toxin. Thus their mode of action would appear to be more specific.

Promethazine protected against the fatty liver caused by macrozamia in rats kept at ambient room temperature but failed to protect against that induced by CCl_4 or DMN[4]. However the drug was administered at more frequent intervals in this study than by Rees and co-workers.[4]

The effect of macrozamia in producing necrosis and a fatty liver would not appear to be directly related to changes in hepatic ATP levels. ATP levels in the liver are unaltered early after macrozamia poisoning and the protective agents are effective despite striking depressions of ATP levels in rats kept at ambient toom temperature.

Promethazine stimulated an increase in the rate of release of β-glycerophosphate phosphatase from LM fractions despite its protective effect against the necrotizing action of macrozamia kernels. Other "antihistamines" have been shown to labilise lysosomal membranes at high concentrations in *vitro*.[15] These observations support previous results[1] indicating that such an increase in the rate of release of β-glycerophosphate phosphatase in *vitro* does not necessarily indicate *in vivo* activation of lysosomal enzymes.

TABLE 3. LIVER LIPID CONTENT AND RELEASE OF β-GLYCEROPHOSPHATE PHOSPHATASE FROM LM FRACTIONS FROM LIVERS

Treatment	Experiment 1			Experiment 2	
	Free β-glycerophosphate phosphatase activity as a % of total			Liver lipid content mg lipid/g liver wet weight	
	Minutes of preincubation				
	15	45	75		
Control	15	33	62	43	—
Adenosine	23	44	64	51	—
Macrozamia	23	58	93	66	—
Adenosine + macrozamia	20	36	63	53	—
Control	—	—	—	—	44
Promethazine	—	—	—	—	50
Macrozamia	—	—	—	—	103
Promethazine + macrozamia	—	—	—	—	93

System: As in previous paper.[1] Rats kept in hot room at 37°. Rats in experiment 1 were destroyed 4 hr after poisoning; those in Experiment 2 were destroyed 12 hr after poisoning. Results are means of four rats.

TABLE 4. GLUTAMATE OXIDATION BY LIVER MITOCHONDRIA

Promethazine dosage	Animal Treatments			Promethazine + macrozamia
	Control	Promethazine	Macrozamia	
25 mg/kg 0 hr 12·5 mg/kg 4 hr	100	103	73	79
50 mg/kg 0 hr. 25 mg/kg 4 hr	100	98	68	68
50 mg/kg 0 hr 25 mg/kg 5 hr	100	105	78	98

System: As previously described;[1] Rats poisoned for 6 hr. Results are means of two experiments, and are expressed as a percentage of control (Control oxygen uptake/mg mitochondrial N/20 min was 120 μl).

Hypothermia would not appear to be an important factor in the protective action of adenosine against the very early effects of macrozamia poisoning. However, the toxicity of adenosine to rats kept in the hot room precluded further investigation of this subject.

The protective effect of promethazine against macrozamia poisoning appears to be dependent upon body temperature. The elevated body temperature of rats kept in the hot room may permit very rapid metabolism of the drug, lowering the concentration below that necessary for the protective effect. It is possible that the protection afforded by promethazine against mitochondrial changes may be an *in vitro* effect by virtue of its high concentrations in the homogenate prepared from the livers.

Recent reports have shown that methylazoxymethanol, the active component of the cycad azoxyglycosides, cycasin and macrozamine, acts as an alkylating agent.[16,17] The failure of cysteine or cystaeamine to ameliorate the toxic effects of macrozamia may be related to the rapid rate of metabolism of the agents *in vivo*.[18,19] Lipid peroxida-

tion would not appear to play an important role in the pathogenesis of macrozamia poisoning as the antioxidant, DPPD, failed to diminish the toxic effects of macrozamia kernels.

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