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Acute toxicity of aristolochic acid in rodents*

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Abstract. The acute toxic effects of aristolochic acid (AA) were tested in rats and mice of both sexes. Oral or intravenous administration in high doses was followed by death from acute renal failure within 15 days. Histologically, the predominant features were severe necrosis affecting the renal tubules, atrophy of the lymphatic organs and large areas of superficial ulceration in the forestomach, followed by hyperplasia and hyperkeratosis of the squamous epithelium. The LD_{50} ranged from 56 to 203 mg/kg orally or 38 to 83 mg/kg intravenously, depending on species and sex.

Key words: Aristolochic acid – Toxicology – Acute toxicity – Rat – Mouse

Introduction

The toxicology of aristolochic acid (AA) has attracted considerable attention since the discovery of its carcinogenic (Mengs et al. 1982; Mengs 1983) and mutagenic properties (Schimmer et al. 1982; Abel and Schimmer 1983; Frei et al. 1983; Manolache et al. 1984; Puri and Müller 1984; Abel 1985; Frei et al. 1985; Schmeiser et al. 1985, 1986). However, little information is available regarding the general toxicity of AA. The relevant literature contains only a few statements regarding nephrotoxic (Martincic 1956; Méhes et al. 1958; Hedwall 1961; Peters and Hedwall 1963; Jackson et al. 1964; Thiele et al. 1967), cytostatic (Kupchan and Doskotch 1962; Kupchan and Merianos 1968; Möse 1975; Schvartzman et al. 1977; Moretti et al. 1979) and antifertility effects (Pakrashi and Chakrabarty 1978; Pakrashi et al. 1980).

To enlarge our knowledge of the toxicological profile of AA, its acute toxic effects were investigated in rodents. The results are reported below.

Materials and methods

Animals and environment. The experimental animals were NMRI mice (Süddeutsche Versuchstierzuchtanstalt GmbH & Co., Tuttlingen, FRG) and Wistar rats (Mus Rattus GmbH & Co., Brunnthal, FRG) of both sexes, having body weights of 20 and 200 g, respectively.

The animals were housed in plastic cages (Makrolon type III) under conventional laboratory conditions at a room temperature of 22 ± 1 °C and a relative humidity of $50 \pm 10\%$. They were given standardized pellet food (ssniff R, Versuchstier-Diäten GmbH, Soest, FRG) and drinking water as required. Food was withdrawn for approximately 16 h before oral administration. The acclimatization time was 7 days.

Compound and treatment. AA was given in a single dose as the sodium salt (mixture of 77.24% AAI and 21.18% AAII %; Dr. Madaus GmbH & Co., Cologne, FRG) through a rigid gastric tube or intravenously into a tail vein. The dose ranges tested are set out in Table 1. The drug was dissolved in distilled water or physiological saline. The volumes in which it was administered were 10 or 20 ml/kg, depending on mode of administration and species. To ascertain dosage in each individual the animals were weighed immediately before treatment.

Observations. All the rats and mice were kept under observation for the whole day after administration of the drug and were then inspected at least once daily for up to 21 days thereafter. Weighing was done on 5 days per week for 3 weeks.

Post-mortem examinations. Necropsies were carried out as soon as possible after death on all rats and mice which died during the course of the trial. After the macroscopic findings had been recorded, the heart, lungs, thymus, spleen, liver, stomach, duodenum, kidneys, adrenals, testes, epididymes and ovaries were fixed in formalin for histological processing. Paraffin and frozen sections were stained with haematoxylin and eosin or scarlet red, respectively.

Statistics. LD_{50} values were calculated from the mortality data from each dose group by means of probit analysis (Miller and Tainter 1944).

Results

Clinical signs

Administration of AA in large single doses by the intragastric or intravenous routes was followed within a few days

* Dedicated to Dr. Rolf Madaus on the occasion of his 65th birthday.

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Table 1. LD_{50} values in rats and mice after oral and intravenous administration of AA

Species	Sex	n^a	Mode of administration	Dose range mg/kg	LD_{50} mg/kg	Survival time days
Rats	♂	10	p.o.	120–295	203.4	4–12
	♀	10		150–300	183.9	5–10
	♂	10	i.v.	62–110	82.5	1–10
	♀	10		38–86	74.0	4–8
Mice	♂	10	p.o.	10–70	55.9	1–15
	♀	10		60–120	106.1	1–15
	♂	10	i.v.	17–102	38.4	1–13
	♀	10		40–125	70.1	1–8

[a] Number of animals per dose group

by dose-dependent reactions in rats and mice of both sexes. These consisted of sedation, piloerection, abnormalities of coordination, dyspnoea, kyphotic posture and occasionally tremor. In the terminal stages the animals adopted the prone position and were totally apathetic. The animals died within 15 days, the interval depending on the dose. Clinically, the behaviour of the survivors had reverted almost entirely to normal by this time.

After treatment with AA the rats and mice lost up to 22% of their body weight within 15 days. In most cases they did not regain their original weight by the end of the experiment.

Mortality rates

The LD_{50} values for rats and mice are set out in Table 1. The figures suggest that AA is slightly more toxic to mice than to rats. The highest LD_{50} after intragastric administration was 203 mg/kg for male rats, while the lowest figure was 56 mg/kg for male mice. The corresponding LD_{50} values for intravenous injection were 83 mg/kg for male rats and 38 mg/kg for male mice. The LD_{50} values for male mice were lower by a factor of approximately 2 than those for females. There were no differences between male and female rats.

Post-mortem examinations

The macroscopic and microscopic changes were largely independent of the mode of administration or species, and are hence described collectively below.

Macroscopical findings. The predominant necropsy finding among rats and mice dying up to 15 days after treatment was atrophy of lymphoid organs. The kidneys were slightly enlarged and were conspicuously pale, as was the liver surface. The cut surfaces of the kidneys showed a yellowish cortex of soft consistency. Among those animals which died within 5 days after oral administration of AA, the forestomach showed severe inflammation and the entire mucosa had separated. The stomach as well as the small intestine contained massive amounts of blood clots. Those animals which died at a later stage showed small focal erosions in the forestomach, together with definite thickening of its wall.

Histological findings. The organs of the lymphoid system – spleen, thymus and any lymph nodes fortuitously included in the histological preparations – showed dose-dependent lymphocyte depletion due to cell destruction (Fig. 1). In the kidneys there was extensive tubular necrosis

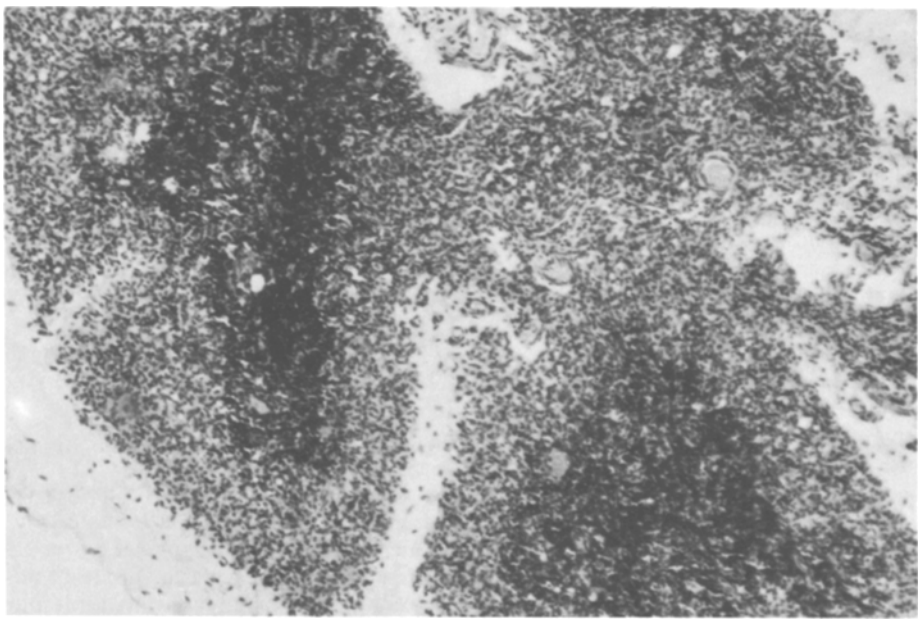

Fig. 1. Moderate lymphocyte depletion in the cortex of the rat thymus 6 days after oral treatment with 200 mg/kg AA. Magnification: 50 x, H. E.

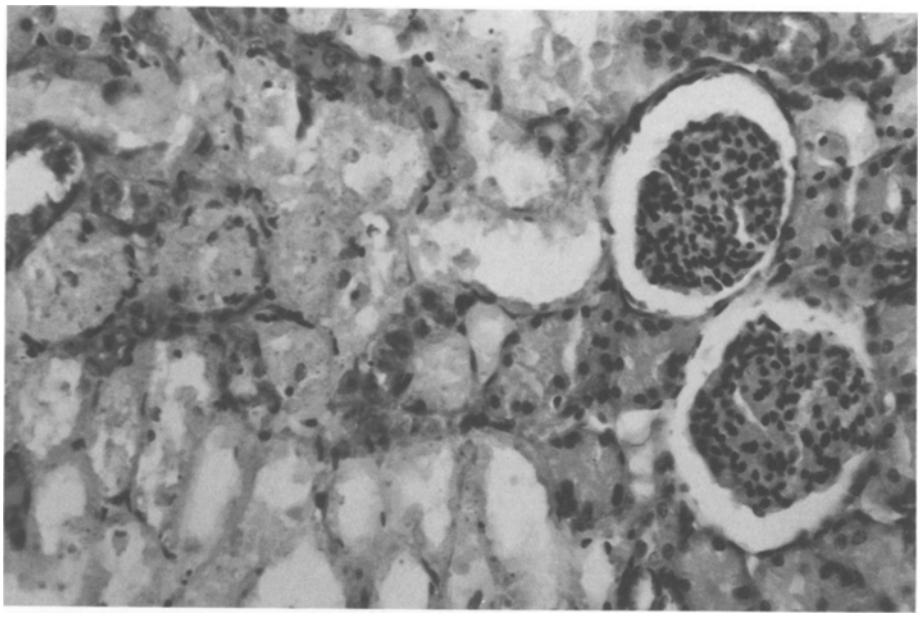

Fig. 2. Severe tubular necrosis of the rat kidney 6 days after oral treatment with 200 mg/kg AA. Magnification: 250 x, H. E.

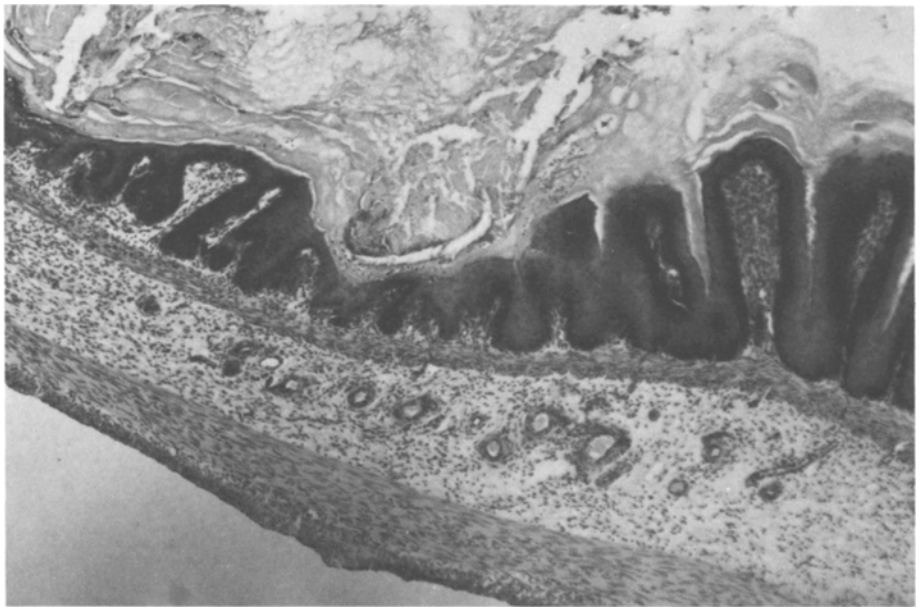

Fig. 3. Hyperplasia and hyperkeratosis of the rat forestomach epithelium with mononuclear cell infiltration and edema in the submucosa 14 days after oral treatment with 200 mg/kg AA. Magnification: 50 x, H. E.

throughout the cortex, involving practically every nephron included in the sections (Fig. 2). Masses of cell debris and protein casts were present within the renal tubules, more especially in the collecting tubes of the medulla. The changes in the adrenals consisted of loss of the normal sudanophilia of the cortex, together with pyknosis of some nuclei and single cell necrosis. Regressive changes were also found in the liver and in the duodenum. In the testes spermiogenesis was severely curtailed. Regressive changes in the germinal epithelium were also noted in a few instances. The tubules of the epididymes contained degeneration products of epithelial cells and only a few sperms.

After intragastric administration of AA the forestomach showed large areas of ulceration with almost total loss of the squamous epithelium and dense infiltration by granulocytes. Among those animals which died 10 days after poisoning or later the lesions were healed, and the main changes were hyperplasia and hyperkeratosis of the squamous epithelium (Fig. 3). In a few animals the glandular mucosa of the stomach showed superficial gastritis of minor degree. In animals which had received the drug intravenously there was distinct atrophy of the mucosa of the forestomach, and in some cases of the glandular mucosa as well. Severe necrotic lesions of the hepatic parenchyma were noted after intravenous administration of AA, particularly in mice.

The lungs, heart and ovaries showed no changes which could be attributed to the treatment with AA.

Discussion

On administration in large single doses to rats and mice, AA causes severe lesions, both local (oral administration) and systemic, the kidneys being the toxicological target organ in both species. Irrespective of species or mode of administration, the renal cortex showed confluent tubular

necrosis of extreme degree. In view of the severity of these lesions it seems reasonable to assume that animals died of uraemic coma due to acute renal failure. These findings provide satisfactory confirmation of the statements made in earlier studies (Martincic 1956; Méhes et al. 1958; Hedwall 1961; Peters and Hedwall 1963; Jackson et al. 1964; Thiele et al. 1967).

In view of the severe renal damage, secondary effects on other organs were to be expected. The regressive changes, which were particularly striking in spleen and thymus, are hence interpreted as secondary toxic changes due to uraemia. It may be surmised that the functional inadequacy of the lymphoid organs was an additional factor having adverse effects on survival.

The local tissue lesions in the forestomach which followed oral administration had previously been observed in earlier trials with considerably lower doses (Mengs 1983). One striking feature is the rapid and excessive regeneration, which led to hyperplasia and hyperkeratosis of the squamous epithelium. Continued treatment with AA is known to produce papillomas within a few weeks, subsequently followed by keratinizing carcinomas of the squamous epithelium, on the basis of this rapidly proliferating epithelium (Mengs et al. 1982; Mengs 1983).

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