PHARMACOLOGY

Vitamin B_6 as an Antidote against the Rodenticide 'Castrix' (2-Chloro-4-methyl-6-dimethylaminopyrimidin)

THE 'Castrix' molecule (2-chloro-4-methyl-6-dimethylaminopyrimidin)[1,2] resembles the vitamin B_6-antagonist toxopyrimidin. In rodents poisoned with this rodenticide the symptoms are an initial depression followed in 40–60 min by a 'search and flight reaction', spasticity, and tonic-clonic seizures, often with tongue-bite. Sound and touch aggravate the severity of the seizures, which may end with death from respiratory failure, dependent on dosage. Survival for more than 5 h usually results in total recovery within 24 h. As these symptoms are almost identical with those seen after administration of certain carbonyl trapping agents and vitamin B_6-antagonists[3–6] we tried vitamin B_6 as an antidote.

A total of 540 mice were used for the experiments. In the strain of mice utilized the LD_{50} of 'Castrix' when administered subcutaneously was 1·3 mg/kg in males and 1·1 mg/kg in females. On oral administration the LD_{50} was 1·2 mg/kg in female mice.

In all experiments pyridoxin, pyridoxal or pyridoxal phosphate was administered subcutaneously in a dosage of 25 mg/kg, which dosage was chosen arbitrarily. Mice injected subcutaneously with twice the LD_{50} of 'Castrix' showed no symptoms when pyridoxin was injected simultaneously with the 'Castrix', while untreated controls showed almost 100 per cent mortality. In another experimental series 'Castrix' was injected intraperitoneally at a dosage of 2·5 mg/kg. The first muscular symptoms, spastic gait and raised tails, appeared in about 30 min, at which time half the animals were treated with pyridoxin. Among the treated animals no seizures were seen, and all symptoms of toxicity disappeared within 10 min. All mice in the control group died within 90 min. Pyridoxal and pyridoxal phosphate were found to have the same effect as pyridoxin, while thiamine, nicotinamide, riboflavin, glutamic acid, and pyruvic acid had no effect. The last two compounds were tested both separately and in combination.

As a further test of the antidotal effect, the LD_{50} was determined in 125 female mice pretreated with pyridoxal immediately before the intraperitoneal injection of 'Castrix'. In these circumstances the LD_{50} was found to be 137 mg/kg. At this high dosage no epileptiform seizures were seen. The only symptoms were somnolence, prostration, and a slight opisthotonus preceding death.

O. KARLOG
E. KNUDSEN

Department of Pharmacology and Toxicology,
Royal Veterinary and Agricultural College,
Copenhagen.

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Amphotericin *B* in the Chemotherapy of Experimental *Schistosomiasis mansoni* in Swiss Mice

IN May 1963, Gordon, St. John and Olsen[1] reported the prolongation of life in Swiss mice experimentally infected with a Puerto Rican strain of *Schistosoma mansoni*, when treated for 5 days with 'Fungizone' (amphotericin *B* made soluble with deoxycholate; E. R. Squibb). In the foregoing experiment, treatment began at a level of 0·5 mg of 'Fungizone'/kg body-weight of mouse a day. The dosage was increased in two daily increments of 0·5 mg/kg to a final level of 1·5 mg/kg a day, which was maintained for three days. Treatment began twelve weeks after infection (approximately 6 weeks after the parasite had begun producing ova). By one week subsequent to the termination of treatment, 80 per cent of the untreated but only 27 per cent of the treated animals had died. A χ^2 test showed that the experimental results fit to a confidence limit of 98 per cent the hypothesis that treatment influenced survival.

The work described here was undertaken to demonstrate whether the observed prolongation of life was due to a reduction in the number of worm pairs per mouse (that is, a reduction of infection-level due to treatment with 'Fungizone').

The mice used in this experiment were strain *CD*–1 Swiss, obtained from Charles River Breeding Laboratories, Inc. The snails used to obtain cercariæ were laboratory-reared *Australorbis glabratus*, obtained from several sources. Both the snails and the parasites were of Puerto Rican origin.

Large numbers of snails were induced to shed cercariæ by placing them in artificial pond water, in a shallow 'Pyrex' baking pan, and exposing them to a fluorescent lamp at close range. By this method, a concentration of 100 cercariæ/ml. was obtained.

Mice were infected by intraperitoneal injection of 1 c.c. of the above-described cercariæ-water per mouse. Treatment with 'Fungizone' began 13 days after infection. Treated animals comprised four groups, receiving daily doses of 1·0, 1·5, 2·0, and 2·5 mg of 'Fungizone'/kg mouse for 40 days. A fifth group received no treatment of any kind subsequent to infection. Treatment in all groups began at the 0·5-mg/kg level and increased in daily increments of 0·5 mg/kg until the final levels were reached. For the first seven days, all treatment was intravenous, thereafter intraperitoneal. The animals were killed within five days after terminating the treatments. The individual infection-levels of all animals were determined by counting the number of viable worms per mouse. The worms in the mesenteric venules were counted by carefully searching these structures at a magnification of ten times with a dissecting microscope. Those which remained in the liver were counted by crushing this organ flat between two glass plates and carefully searching at a magnification of 10 times. No parasites were found associated with any other organ or system of the mice.

Statistical methods by which treatment groups were compared included a one-way analysis of variance for unequal sample sizes, and a calculation of the value of Student's t for each treatment group compared with the group that received no treatment.

Table 1 presents the treatment groups and the mean number of worms per group (that is, mean infection-level per group) with the standard deviation of each group mean.

Table 1

Group No.	Daily dosage 'Fungizone'	No. mice in group	Mean level infection	Standard deviation
A	none	11	32·7	16·9
B	1·0 mg/kg	12	30·8	20·2
C	1·5 mg/kg	19	18·9	11·4
D	2·0 mg/kg	9	18·1	9·7
E	2·5 mg/kg	10	14·6	6·4

An analysis of variance comparing the infection-levels for all groups is presented in Table 2.

Table 2

Source of variation	Degrees of freedom	Sum of squares	Mean square (variance estimate)
Between samples	(5—1) = 4	(*SSB*) 3,025·74	$MSB = \frac{SSB}{4} = 756{\cdot}435$
Error	(61—5) = 56	(*SSE*) 10,305·67	$MSE = \frac{SSE}{56} = 184{\cdot}048$
Total	(61—1) = 60	13,331·41	

$$F = \frac{MSB}{MSE} = \frac{756{\cdot}435}{184{\cdot}048} = 4{\cdot}01$$