EFFECT OF SUBSTITUTED MERCAPTOPURINES ON THE RAT LITTER *IN UTERO*

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Summary. 6-Amyl, 6-propyl, and 6-benzyl, 2-amino-6-benzyl and 6-(2'4' dinitrophenyl)-mercaptopurine (MP) were examined for the effect on the rat litter *in utero*. The toxicity of the LD₅₀ for the mother rat varied from 300 to more than 500 mg/kg. Complete litter destruction with the compounds was possible on the 7th day of gestation with doses tolerated by the mothers. The best ratios between LD₅₀ to the mother and the lethal dose to the litter of 1 : 12 were obtained with 6-(2'4' dinitrophenyl) and of 1 : 10 with 2-amino-6-benzyl-MP. This was followed by 6-propyl and 6-benzyl-MP with a ratio of 1 : 5, followed by 6-amyl-MP with a ratio of 1 : 2.5. The findings indicate that none of the compounds achieved as favourable a ratio between LD₅₀ to the mother and litter destruction as 6-thioguanine with 1 : 30.

INTRODUCTION

In previous work, (Thiersch, 1954, 1957), it was established that 6-mercaptopurine, 6-chloropurine and especially 6-thioguanine would destroy the foetuses and litters of the rat *in utero* with doses well tolerated by the mothers. In the present study, an attempt was made to detect other substituted thiopurines which might have a better ratio between the LD₅₀ to the mother animal and the lethal dose to the litter.

MATERIALS AND METHOD

The compounds, supplied by G. Hitchings with toxicity data, are listed in Table 1. The materials are less toxic than 6-mercaptopurine and are in the range of toxicity of 6-chloropurine or 6-thioguanine. The materials were dissolved in distilled water and given orally or intraperitoneally. The Long-Evans strain of rats was used entirely in the present experiments. The procedure followed was previously outlined in the 6-mercaptopurine paper (Thiersch, 1954). The onset of pregnancy was determined in the animals by the finding of masses of spermatozoa or a solid plug in the vagina. The animals were then separated into single stainless steel cages where urinary and faecal contact was impossible. The mother rats were then injected at different periods of their gestation and sacrificed on the 21st day of gestation under ether anaesthesia. The uteri were then removed, the uterine contents noted and recorded, the

surviving placentas and foetuses measured and weighed. The skeletons of surviving foetuses were cleared with oil of wintergreen to study possible malformation and stained with alizarin. The results of the experiments were tabulated and are seen in Table 1. Malformations were not induced in any surviving foetuses.

TABLE 1
EFFECT OF SUBSTITUTED MERCAPTOPURINES ON THE RAT LITTER *in utero*

Compound	Single LD ₅₀ (mg/kg i.p.)	Complete litter destruction 7th gestation day (mg/kg dose)	Ratio LD ₅₀ to mothers Lethal litter dose	Dose 11th gestation day (mg/kg)	Resorbed foetuses (%)
6-Chloropurine	500	100	1 : 5	100	14
6-Thioguanine	300	10	1 : 30	10	75
6-Mercaptopurine	200	30	1 : 6	30	5
6-Amyl-MP	>500	200	1 : 2.5	200	20
6-Propyl-MP	>500	100	1 : 5	150	6
6-Benzyl-MP	>500	75	1 : 5	50	7
2-Amino-6-benzyl-MP	>500	100	1 : 10	50	—
6-(2'4' Dinitrophenyl)-MP	>300	50	1 : 12	100	7
6-1'-Methyl, 4'-nitro, 5'-imidazolyl-MP	300	10	1 : 30	20	2
2-Amino-6-1'-methyl, 4'-nitro, 5'-imidazolyl- MP	>300	5	1 : 40	20 oral 50	37 oral 30

Summary of results of substituted mercaptopurines tested for effect on rat litter *in utero*. 6-Chloropurine, 6-thioguanine (Thiersch, 1954) and 6-MP (Thiersch, 1957) are compared with heterocyclic substituted MP. Note maximal effect on litter immediately after implantation (7th day of gestation) and insensitivity at mid-term (11th day of gestation).

TABLE 2
6-AMYL-MP. LD₅₀ GREATER THAN 500 MG/KG I.P.

Dose (mg/kg i.p.)	Day of dose (post coitum)	No. rats	Total implantations	Foetuses					Mothers with entire litter resorbed (%)
				Total implantations (%)					
				Normal	Stunted	Dead	Completely resorbed	Surviving placentas only	
50	7+8	10	111	66	0	2	22	10	20
50	11+12	17	186	99	0	0	0	1	0
100	7	13	142	15	5	0	53	27	61.5
200	7	11	126	0	0	0	100	0	100
200	11	9	88	67	7	0	16	10	11.1

RESULTS

6-Amyl-MP

LD₅₀ greater than 500 mg/kg intraperitoneally (i.p.) (Table 2). The table shows that a single 100-mg dose given on the 7th day of gestation reduced the number of surviving foetuses to 20% and destroyed completely eight out of thirteen litters in the group of rats tested. The 200-mg/kg dose did not permit survival

of a single foetus. From Table 2, it is evident that the ratio between the LD₅₀ to the mother and the lethal dose to the litter is approximately 1 : 2.5.

6-Propyl-MP

Single LD₅₀ greater than 500 mg/kg i.p. (Table 3). In doses of 50 mg/kg i.p.

TABLE 3
6-PROPYL-MP. LD₅₀ GREATER THAN 500 MG/KG I.P.

Dose (mg/kg)	Day of dose (post coitum)	No. rats	Total implantations	Foetuses					Mothers with entire litter resorbed (%)
				Total implantations (%)					
				Normal	Stunted	Dead	Completely resorbed	Surviving placentas only	
50 i.p.	7+8	13	137	2	4	0	88	6	77
50 i.p.	11+12	3	34	82	12	0	3	3	0
5 oral	11+12	9	90	92	6	0	1	1	0
10 i.p.	7+8	11	102	55	10	0	23	12	18.2
50 oral	7+8	14	132	6	1	0	88	5	78.5
100 oral	7	11	112	0	0	0	99	1	100
150 oral	11+12	14	149	79	14	1	3	3	0

given on the 7th and 8th day of gestation, this compound permitted only the survival of 6% of the foetuses and completely destroyed ten out of thirteen litters. Most of the surviving foetuses were stunted. The same dose given i.p. or even 150 mg/kg orally on the 11th and 12th day of gestation was not effective, permitting the survival of 94% of all foetuses. The 50-mg/kg oral doses given on the 7th and 8th day had the same results as the i.p. doses. The 100-mg doses given orally on the 7th day destroyed all litters and foetuses. From Table 3, it is evident that the LD₅₀ to the mother and the litter-destroying doses was approximately 1 : 5.

TABLE 4
6-BENZYL-MP. LD₅₀ GREATER THAN 500 MG/KG I.P.

Dose (mg/kg)	Day of dose (post coitum)	No. rats	Total implantations	Foetuses					Mothers with entire litter resorbed (%)
				Total implantations (%)					
				Normal	Stunted	Dead	Completely resorbed	Surviving placentas only	
50 i.p.	5	9	83	83	0	0	15	2	0
50 i.p.	7	13	128	20	0	0	68	12	77
50 i.p.	11	11	101	88	0	5	0	7	0
100 oral	7	13	136	0	0	0	95	5	100
50 i.p.	6	5	49	86	0	0	7	7	0

6-Benzyl-MP

Single LD₅₀ greater than 500 mg/kg i.p. (Table 4). In doses of 50 mg/kg i.p. given on the 7th day the compound permitted the survival of 20% of all the foetuses and completely destroyed ten out of thirteen litters. The same dosage given on the 11th day of gestation i.p. had only a minor effect on the foetuses. A

100-mg oral dose given on the 7th day of gestation destroyed all fetuses and litters. This gives a ratio of LD₅₀ to the mother and lethal dose to the litter of approximately 1 : 5.

2-Amino-6-benzyl-MP

Single LD₅₀ greater than 500 mg/kg (Table 5). A 100-mg oral dose given on

TABLE 5
2-AMINO, 6-BENZYL-MP. LD₅₀ MORE THAN 500 MG/KG I.P.

Dose (mg/kg)	Day of dose (post coitum)	No. rats	Total implantations	Foetuses					Mothers with entire litter resorbed (%)
				Total implantations (%)					
				Normal	Stunted	Dead	Completely resorbed	Surviving placentas only	
20 i.p.	5	10	84	80	1	0	14	5	10
20 i.p.	7	11	100	22	7	0	59	12	45.5
50 i.p.	5	13	135	84	3	0	7	6	0
50 i.p.	7	18	192	0.6	0	0	93.4	6	89
50 i.p.	9	9	93	89	0	0	2	9	0
50 i.p.	10	10	105	95	0	0	3	2	0
50 i.p.	11	10	103	100	0	0	0	0	0
100 oral	7	12	112	5.5	0	0	84.5	10	83.3

TABLE 6
6-(2'4' DINITROPHENYL)-MP. LD₅₀ GREATER THAN 300 MG/KG I.P.

Dose (mg/kg i.p.)	Day of dose (post coitum)	No. rats	Total implantations	Foetuses					Mothers with entire litter resorbed (%)
				Total implantations (%)					
				Normal	Stunted	Dead	Completely resorbed	Surviving placentas only	
50	7+8	12	140	0	0	0	100	0	100
10	11	10	110	93	1	0	5	1	0
10	7	13	147	61	10	0	19	10	77
2	7+8	15	141	96.5	2	0	0	1.5	0
25	7	14	138	0	0	0	93	7	100
50	7	13	155	0	0	0	94	6	100
50	11	10	89	100	0	0	0	0	0
100	11	12	119	83	20	2	3	2	0

the 7th day of gestation destroyed 94% of all foetuses and ten out of twelve litters completely. The 20-mg/kg i.p. dose given on the 7th day of gestation destroyed 71% of all foetuses and stunted seven of the survivors. However, only five of eleven litters were entirely destroyed. The 50-mg-i.p. dose given on the 7th day of gestation to a group of eighteen rats permitted the survival of less than 1% of all the foetuses and completely destroyed sixteen out of eighteen litters.

From this, it is evident that amination in the 2 position on the molecule increased the effect on the foetuses. The data shows a ratio of 1 : 10 between the LD₅₀ to the mother and complete litter destruction.

6-(2'4' Dinitrophenyl)-MP

Single LD₅₀ greater than 300 mg/kg i.p. (Table 6). A single 25-mg/kg dose

given i.p. on the 7th day of gestation destroyed all litters and foetuses. In contrast to this, the 100-mg/kg i.p. dose given on the 11th day had hardly any effect on the life of the foetuses, but stunted 20%. This gave a ratio of LD₅₀ to the mother and lethal dose to the litter of 1 : 12.

DISCUSSION

From the findings listed in the Tables, it is evident that the most efficient compound in litter destruction was the 6-(2'4' dinitrophenyl)-MP followed by the 2-amino-6-MP. It is evident that the effect of the compounds was most marked on the 7th day of gestation as soon as implantation was completed. Neither before or later in foetal life were similar results obtained. On the 11th day of gestation, even multiple doses, sufficient to destroy the entire litters in groups of animals on the 7th day of gestation, had only minor effects on the litter *in utero*. This confirms previous observations with 6-mercaptopurine, 6-chloropurine and 6-thioguanine.

Histological examinations of the spleen, liver, kidney, adrenals and bone marrow of the mother animals showed no evidence of bone-marrow depression or lesions that could be histologically called significant. The bone-marrow depression which followed injection of the animals had subsided and the bone marrow had completely recovered at the time of sacrifice.

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