Antimitotic Activity of a Steroid Guanylhydrazone

I HAVE previously shown that a guanylhydrazone derivative of a known steroid molecule is a powerful antimitotic agent[1]. This compound, the structure of which is shown in Fig. 1, is 3-guanyl-hydrazone-androstan-17-ol; it is listed by the Italian pharmaceutical house Vister as 2052 *V* (Fig. 1).

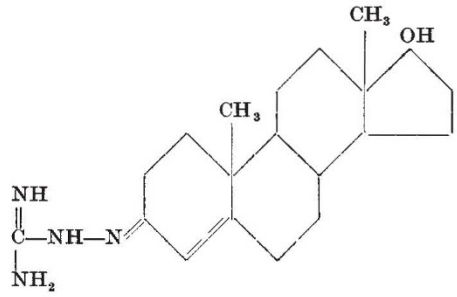

Fig. 1. 2052 *V*, 3-guanyl-hydrazone-androstan-17-ol

The present investigations were made in order to characterize the antimitotic activity of 2052 *V*, *in vitro* (in human amnion cell lines and primary kidney cell cultures of pigs and hamsters) and *in vivo* (in mice and hamsters).

Human amnion cell cultures (H. A. Mascoli's line), propagated *in vitro* in Hanks medium, supplemented with lactalbumin hydrolysate and 5 per cent calf serum, were collected by trypsinization from cultures which had been stationary for 36 h, dispersed in the same medium (300,000 cells/ml.) and seeded on coverslips which had been placed on the bottom of Petri dishes; at the same time either 2052 *V* (1 and 10 μg/ml.) or colchicine (1 μg/ml.) were added. Untreated cultures were kept as controls. At intervals of 12, 24, 36 and 48 h the coverslips were fixed in methanol and stained with Giemsa. Mitotic figures were registered and the mitotic index calculated.

Table 1. ANTIMITOTIC ACTIVITY OF 2052 *V* IN COMPARISON WITH THAT OF COLCHICINE ON HUMAN AMNION CELL CULTURES

Percentage of mitotic figures and mitotic index (*M.I.*) in cell cultures at different times after treatment.

	No treatment					2052 *V* (1 μg/ml.)					Colchicine (1 μg/ml.)				
h	*M.I.*	P	M	A	T	*M.I.*	P	M	A	T	*M.I.*	P	M	A	T
12	2·5	32	40	8	20	14·8	2	98	—	—	16·2	4	96	—	—
24	2·8	25	34	11	30	46·0	—	100	—	—	52·0	—	100	—	—
36	2·3	16	49	11	24	12·8	—	98	1	1	27·6	—	100	—	—
48	2·1	15	51	9	25	4·0	—	100	—	—	15·0	—	100	—	—

P, Prophase; M, metaphase; A, anaphase; T, telophase.
This is a typical experiment representative of many other experiments.

2052 *V* produces an effect like that of colchicine (Table 1); at a concentration of 1 μg/ml. it produces a complete mitotic block in metaphase, as shown by the disappearance of anaphases and telophases and by the increase of abnormal metaphases. The mitotic index declines faster with 2052 *V* than with colchicine because metaphases arrested by the former compound degenerate more rapidly, and because a few cells may break through the 2052 *V* block. At a concentration of 10 μg/ml. of 2052 *V* a clear cytotoxic effect is present within 24 h. In cultures treated with 2052 *V*, cells in inter-kinesis display nuclear budding which leads to polynucleated cells. This process becomes evident after 12 h of treatment and affects a progressively larger number of cells.

In order to evaluate further analogies between 2052 *V* and colchicine other experiments have been carried out on primary kidney cell cultures of pigs and hamsters. It is known that hamster cells are resistant to the antimitotic activity of colchicine *in vivo*[2,3] and *in vitro*[3]. Either 2052 *V* or colchicine was added to the culture medium at a concentration of 1 μg/ml. Twenty-four hours later mitotic figures were registered and the mitotic index was calculated. Untreated cultures were kept as controls.

As may be seen in Table 2, the kidney cells of pigs are inhibited both by colchicine and by 2052 *V* while the kidney cells of the hamster are resistant to the antimitotic activity of both compounds.

Table 2. INFLUENCE OF 2052 *V* AND COLCHICINE ON PRIMARY KIDNEY CELL CULTURES OF PIG AND HAMSTER

Percentage of mitotic figures and mitotic index (*M.I.*) in cell cultures after 24 h of treatment

	Pig					Hamster				
Treatment	*M.I.*	P	M	A	T	*M.I.*	P	M	A	T
No treatment	1·6	31	42	12	15	1·2	28	42	7	23
2052 *V* (1 μg/ml.)	2·9	—	100	—	—	1·0	20	50	10	20
Colchicine (1 μg/ml.)	3·6	2	97	1	—	1·2	17	69	4	10

2052 *V* was administered intraperitoneally to mice and hamsters in single doses of 10, 25, 50 or 100 mg/kg. Other animals were given colchicine 10 mg/kg intraperitoneally. The animals were killed 8 and 30 h after treatment, bone marrow mitotic stages were registered and mitotic indices calculated.

Table 3. INFLUENCE OF 2052 *V* (100 MG/KG INTRAPERITONEALLY) AND COLCHICINE (10 MG/KG INTRAPERITONEALLY) ON BONE MARROW MITOSES OF MOUSE AND HAMSTER

Percentage of mitotic figures and mitotic index (*M.I.*) in bone marrow cells at different times after treatment. Percentage calculated on the basis of at least three animals.

		Mouse					Hamster				
Treatment	h	*M.I.*	P	M	A	T	*M.I.*	P	M	A	T
No treatment	—	0·8	20	40	10	30	0·9	9	47	18	26
2052 *V*	8	2·4	—	95	—	5	0·9	10	45	15	30
2052 *V*	30	1·6	20	60	5	15	0·7	5	45	20	30
Colchicine	8	6·6	—	100	—	—	1·5	3	63	13	21
Colchicine	30	5·3	—	95	—	5	1·3	5	50	15	30

The most significant results obtained are reported in Table 3. In mice, 2052 *V* in doses of 100 mg/kg caused a nearly complete disappearance of ana-telophases at 8 h, and also caused a rise of mitotic index from 0·8 to 2·4 per cent. After 30 h these effects regressed. Single doses of 10, 25 and 50 mg/kg were less effective. Colchicine at the dose of 10 mg/kg was more effective than 100 mg/kg of 2052 *V* and its blockade was more persistent.

In hamsters, unlike mice, both 2052 *V* and colchicine failed to affect mitoses significantly.

In conclusion, 2052 *V* is a mitotic poison both *in vitro* and *in vivo*. It shows several analogies with colchicine and griseofulvin. In fact, all three compounds produce a block in metaphase; moreover, like colchicine[2,3] and griseofulvin[7], 2052 *V* is ineffective in hamsters both *in vivo* and *in vitro*. The similarity between griseofulvin and 2052 *V* is more marked because this compound produces nuclear buddings which are identical to those seen with griseofulvin[4-6]. 2052 *V* is effective *in vitro* at concentrations as low as those of colchicine; however, *in vivo* doses greater than those for colchicine are needed to produce the same effect. Also *in vivo* metaphasic blockade by 2052 *V* is less persistent than that by colchicine.

2052 *V* is of great interest because it is the only known steroid molecule endowed with antimitotic activity similar to that of colchicine. The resistance of hamsters to colchicine, griseofulvin and 2052 *V* suggests that, despite the differences in structure, 2052 *V* also acts as a mitotic spindle poison.

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