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Some Effects of Atrazine on Ehrlich Ascites Tumor Cells *In Vitro* and *In Vivo*

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Triazines were first introduced as herbicidal agents in the early 1950's. Simazine is the best known of the family, and its soil persistence makes it useful in long-term weed control. Atrazine is another member of the family and similar to simazine in its herbicidal properties. It may well be less persistent than simazine (BEATLY 1964), but more persistent than certain other triazines such as propazine, ipazine, and chlorazine (SWITZER and RAUSER 1964). Triazine herbicides, especially simazine, are used to effectively inhibit the growth of most common forms of algae in aquariums, ornamental fish ponds, and fountains (ALGI-GON, M.E. Park Enterprises, Inc.).

Atrazine is stable in dilute acids or aklalies at room temperature, and is only slightly soluble in water; these characteristics encourage persistence in the environment. It is used for non-selective weed control on industial or non-cropped land and selective weed control in certain crops (Pesticide Chemicals Official Compedium, 1966). It may be dangerous to certain desirable trees, shrubs, and plants and should be used carefully in areas containing these plants.

Application of 2 lb/acre simazine and atrazine to soil (TALBERT and FLETCHALL 1964), and subsequent analysis of soil samples at intervals for herbicide levels revealed that conditions favorable for the growth of microorganisms resulted in a more rapid loss of the agents. About 8% of the original atrazine and 15% of the original simazine still remained a year after the initial application, while no residues of either could be detected 16 months after such application.

An explantion for the relative insensitivity of certain crops to members of the triazine herbicide family has been given (HARTLEY 1964). Apparently the herbicide enters the crop plant as well as the weed, but the crop plant is able to metabolize or otherwise detoxify the agent used. The author also stated that the crop plants may play a role in removal of triazine herbicides from soil. The triazine herbicides appear to exhibit their effects

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on plants by interfering with photosynthesis (SHEETS 1961). One proposal is that these agents are able to form hydrogen bonds with one or more enzymes involved in the oxidation of water (GOOD 1962). Sufficient information has not been found about the metabolism of triazine herbicides in plants and animals. In some plants, the chlorotriazines undergo hydrolysis to yield the corresponding hydroxytriazines (MONTGOMERY and FREED 1964). To date, the various metabolites beyond the hydroxytriazines have not been identified (FREED and MONTGOMERY 1969).

There is a noticeable void in the literature concerning the effects of atrazine and other triazine herbicides on mammalian cells and organisms. A few studies have been conducted to determine the actions of atrazine on plant cells. In one such study atrazine, carbaryl, endrin, and certain other pesticides induced a significant number of chromosomal aberrations, chiefly chromosomal breakage, in the C_1 generation in root tip cells of barley seedlings (WUU and GRANT 1966). No entry for atrazine was found listed in the Negative Results of the Cancer Chemotherapy Screening Data of the United States Public Health Service. The acute oral LD_{50} value for atrazine in mice is reported as 1,750 mg/kg (Pesticide Chemical Official Compedium, 1966) and in adult white rats as 3,080 mg/kg (ARENA 1974).

Consideration of the paucity of literature concerning the effects of atrazine and other triazine herbicides on mammalian cells and organisms prompted us to investigate the effects of atrazine on Ehrlich ascites tumor cells, including certain *in vivo* and *in vitro* studies.

MATERIALS AND METHODS

Atrazine was provided in highly purified form (99.0%) by the Pesticides Research Laboratory, Perrine, Florida. Thymidine-methyl-^3H, uridine-5-^3H, L-leucine-^{14}C, and sodium formate-^{14}C were purchased from New England Nuclear Corporation. Ehrlich ascites cells were maintained in BALB/c mice which were purchased from Flow Research Animals, Inc. The investigation included the following parameters which have been described in earlier reports as indicated: rates of synthesis of DNA, RNA, and protein (GALE and HYNES 1968, GALE et al. 1967): *de novo* synthesis of RNA purines (GALE et al. 1968, GALE and SCHMIDT 1968); Ehrlich ascites tumor development *in vivo* as determined by total packed cell volume (TPCV) (GALE et al. 1970). DMSO was used as the solvent system for all parameters in the investigation of atrazine. The volume of DMSO solution injected (ip) was 0.1 ml per 40 g body weight of each control or treated mouse. In the *in vitro* experiments (control and treated) the final concentrations of DMSO were 1% (v/v).

TABLE 1

Effect of Atrazine on Development of the Ehrlich Ascites Tumor In Vivo

Dose mg/kg/day[a]	Number of injections[b]	Average weight change (gm.) T/C[c]	Mortality C	Mortality T	Average TPCV[d] T/C, ml.	% inhibition	S.D.[e] T/C	P Value
120	6	-0.7/3.7	0/8	1/8	1.091/1.772	38	0.344/0.250	< 0.0005
134	8	1.0/4.5	0/8	3/8	1.958/2.775	30	0.223/0.535	< 0.01
134	6	0.4/1.8	0/8	2/8	0.963/1.549	38	0.398/0.355	< 0.05
140	6	0.8/5.8	0/9	2/9	1.208/2.000	40	0.306/0.330	< 0.0005

[a]Dimethyl sulfoxide was the vehicle
[b]Injected intraperitoneally, once per day
[c]Treated/Control
[d]Total packed cell volume
[e]Standard deviation of the TPCV of the treated/control groups

TABLE 2

Effect of Atrazine on RNA Purine Synthesis in Ehrlich Ascites Cells In Vivo[a]

Atrazine mg/kg	Hours after Injection[b]	CPM/mg Ba^{++} RNA Guanine	Adenine	% Change Guanine	Adenine	Guanine/Adenine[c]
0	2	4263	1765	---	---	2.41
125	2	5269	1987	+24	+13	2.65
150	2	2437	725	-43	-59	3.36
0	12	2133	1500	---	---	1.42
100	12	2513	2151	+17	+43	1.17
150	12	1261	1538	-41	+ 3	0.82
200	12	553	686	-74	-54	0.81
0	24	1395	882	---	---	1.58
100	24	1361	1049	- 2	+19	1.30
125	24	792	716	-43	-19	1.11
200	24	807	782	-42	-11	1.04
0	24	2560	1866	---	---	1.37
150	24	1434	1443	-44	-23	0.99
0	36	1771	1798	---	---	0.98
100	36	1951	1764	+10	- 2	1.11
150	36	1243	872	-30	-52	1.43
200	36	1489	1589	-12	-12	0.94

[a]Atrazine, in DMSO, at the doses indicated was injected ip to groups of 3-5 mice.
[b]Represents interval between ip injections of atrazine and formate-^{14}C. Cells were removed and processed as described in reference 5.
[c]Represents guanine/adenine ratio.

RESULTS AND DISCUSSION

Effects of Atrazine on Development of the Ehrlich Ascites Tumor In Vivo

The inhibitory effects of atrazine in DMSO on development of

TABLE 3

Effects of Atrazine on the In Vitro Incorporation of Radioactive Precursors into DNA, RNA, and Protein in Ehrlich Ascites Cells[a]

Synthesis of	Sample[b]	Hours of Incubation[c]	Average CPM[d]	Average % of Control
DNA	Control	0[e]	89598	
	Atrazine	0	75868	85
	Control	1.5	41367	
	Atrazine	1.5	28925	70
	Control	3.0	24123	
	Atrazine	3.0	5268	22
RNA	Control	0	269599	
	Atrazine	0	234706	87
	Control	1.5	103778	
	Atrazine	1.5	85222	82
	Control	3.0	55450	
	Atrazine	3.0	35387	64
Protein	Control	0	20683	
	Atrazine	0	18052	87
	Control	1.5	6842	
	Atrazine	1.5	5369	78
	Control	3.0	3771	
	Atrazine	3.0	1767	47

[a]The cells were incubated with the appropriate radioisotopes for periods of 20 minutes, at the end of which the reactions were terminated by the addition of an equal volume of 10% TCA. Radioactive precursors and final activities were: for DNA, thymidine-methyl-^{3}H (10 μCi/ml); for RNA, uridine-^{3}H (10 μCi/ml); and for protein, L-leucine-^{14}C, uniformly labeled (2 μCi/ml).

[b]Concentraion of atrazine, 10^{-4} M in incubation medium.

[c]Time interval between addition of atrazine and addition of appropriate radioisotope to cells.

[d]Average of 5 similar experiments in which duplicate tubes were included for each sample (average of 10 tubes).

[e]Cells were added to incubation tubes containing atrazine and radioisotope.

TABLE 4

Effects of various concentrations of atrazine on the incorporation of radioactively-labeled precursors of DNA, RNA, and protein in Ehrlich ascites tumor cells *in vitro*.[a]

Synthsis of	Incubation with Atrazine at [M][b]	ppm[c]	Average CPM[d]	% Inhibition
DNA	0		41,050	
	10^{-5}	2.2	39,560	4
	4×10^{-5}	8.6	36,145	12
	8×10^{-5}	17.3	36,023	12
	10^{-4}	21.5	29,186	29
	4×10^{-4}	86.3	6,948	83
	8×10^{-4}	172.5	1,018	98
	10^{-3}	215.7	485	99
RNA	0		29,969	
	10^{-5}	2.2	25,981	13
	4×10^{-5}	8.6	24,252	19
	8×10^{-5}	17.3	23,355	22
	10^{-4}	21.5	21,125	30
	4×10^{-4}	86.3	7,781	74
	8×10^{-4}	172.5	2,652	91
	10^{-3}	215.7	1,874	94
Protein	0		10,438	
	10^{-5}	2.2	9,671	7
	4×10^{-5}	8.6	8,419	19
	8×10^{-5}	17.3	7,709	26
	10^{-4}	21.5	5,264	50
	4×10^{-4}	86.3	2,579	75
	8×10^{-4}	172.5	618	94
	10^{-3}	215.7	354	97

[a]Same as footnote a of Table 3.
[b]Cells were incubated with Atrazine for 3 hours prior to addition of radioactively-labeled precursors.
[c]Represents approximate ratio of Atrazine to total solution, expressed in parts per million.
[d]Values represent averages of 6 reaction vessels for each control and 2 vessels for each Atrazine concentration.

the Ehrlich ascites tumor *in vivo* are shown in Table 1. A total of 6 to 8 ip injections of 120-140 mg/kg/day atrazine produced 30-40% inhibition of the tumor development.

Effects of Atrazine on RNA Purine Synthesis in Vivo

Table 2 shows the effects of atrazine on de novo purine synthesis in vivo. Atrazine appeared to inhibit the incorporation of formate-^{14}C, respectively, especially at the higher concentrations used. The inhibitory effects were most prominent following in vivo exposure of the cells to the herbicide for intervals of 2-12h, were clearly observable after a 24-h interval of exposure, and detectable after a 36-h interval of exposure. These results appear to suggest a persistent inhibitory effect on de novo purine biosynthesis in vivo.

Effects of Atrazine on the Incorporation In Vitro of Radioactive Presursors into DNA, RNA, and Protein in Ehrilch Ascites Cells

The effects of atrazine on the incorporation in vitro of radioactive precursors into DNA, RNA, and protein are shown in Table 3. Incubation of the cells for period of 3 h with 10^{-4} M atrazine resulted in an appreciable decrease in the rates of incorporation of thymidine-methyl-^{3}H into DNA and L-leucine-^{14}C into protein, while producing a smaller but still detectable decrease in the incorporation of uridine-5-^{3}H into RNA. These effects were less obvious following shorter incubation periods of 1 and 2 h.

Comparison of Tables 1 and 4 reveals an interesting correlation between in vivo and in vitro studies with atrazine. Daily single injections of 12 mg/kg (≃86.3 mg/kg) atrazine resulted in 83%, 74% and 75% inhibition of the incorporation of thymidine-methyl-^{3}H, and L-leucine-^{14}C into DNA, RNA, and protein, respectively, in vitro (Table 4). A concentration of 8 x 10^{-4} M (≃172.5 mg/kg) produced 98%, 91%, and 94% inhibition of these respective parameters (Table 4). Thus, the effects of atrazine on DNA, RNA, and protein synthesis in vitro appear to be of adequate degree to explain the moderate inhibitory action of the agent on Ehrlich ascites tumor in vivo in mice.

The present report appears to indicate that atrazine may possess pharmacologic properties against mammalian cells and organisms, and appears to point out the need for additional studies designed to determine the effects of these herbicides on a number of biochemical and pharmacological parameters in variety of mammalian cellular systems.

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