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Chemotherapy of Leukemia VII. Effect of Substituted Triazenes on Transplanted Mouse Leukemia.* (22273)

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During routine screening of compounds for possible antitumor effects, a member of a new series of compounds not previously known to possess chemotherapeutic activity, 3,3-dimethyl 1-phenyl triazene (Triazene I), was found by Clarke *et al.*(1) to inhibit growth of Sarcoma 180 in mice. Following this Dagg, Karnofsky *et al.*(2) demonstrated teratogenic effects on the chick embryo and inhibition of growth of Sarcoma 180 and certain human tumors implanted on the chorioallantoic membrane. An evaluation of chemotherapeutic effects of this and related compounds against various strains of transplanted leukemia in mice has been undertaken and the results are herewith reported.

Method. The general technic used for evaluating chemotherapeutic activity of a given drug by means of its ability to prolong survival time of mice with transplanted leukemia has been described previously(3). The 60th to 70th transplant generations of leukemia 82(4), which originated as a spontaneous leukemia in a C58 mouse in October 1953, were used for most of these studies. This transplantable leukemia usually kills the mice in 10 to 15 days after inoculation, with an elevation of total leukocyte count to the 50000 to 200000 level, enlargement of liver and spleen, and some lymphadenopathy. A few studies were conducted on the 10th to 14th generation of leukemia 5471, which arose as a spontaneous leukemia in a C58 mouse in July, 1954. Although morphologically these 2 leukemias are very similar, 82 has always been relatively refractory to folic acid antagonists, whereas 5471 is quite sensitive. In these experiments mice of the F1 hybrid generation of C58 male X Bagg albino female cross were each injected intraperitoneally with 0.1 cc of saline suspension of minced spleen containing one million cells per inoculum. The A-methopterin sensitive (L1210S) and A-methopterin dependent (L1210AD) variants of Leukemia L1210 obtained from Law(5,6) were also used. These leukemias were transplanted by subcutaneous injections into dba mice of minced tumor tissue suspended in isotonic saline. Treatment was initiated 24 hours after inoculation and repeated 3 times weekly for 10 doses over a 23-day period. The mice were observed for development of leukemia and autopsied at death. Triazenes that have been adequately tested and their maximum tolerated doses are listed below. Because of low solubility in water, these compounds were either dissolved in peanut oil (PO) or suspended in 0.5% sodium carboxymethylcellulose (CMC) in isotonic saline as indicated:

I.	3,3-dimethyl-1-phenyl triazene	12-25 mg/kg (PO)
II.	3,3-diethyl-1-phenyl triazene	150 mg/kg (PO)
III.	3,3-dimethyl-1-p-nitrophenyl triazene	250 mg/kg (CMC)
IV.	3,3-dimethyl-1-p-tolyl triazene	40-62.5 mg/kg (CMC)

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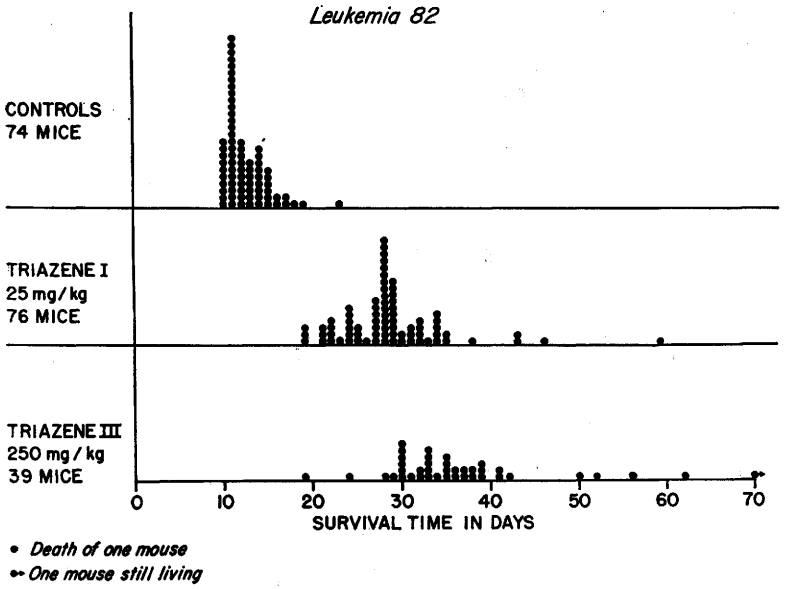

FIG. 1.

Results. The scatter diagram in Fig. 1 shows the relative effectiveness of triazenes I and III in leukemia 82. The mean survival time of the 74 control animals was 12.5 days whereas mice treated with Triazene I at a dose of 25 mg/kg of body weight showed mean survival time of 28.9 days and those receiving Triazene III at 250 mg/kg 35.8 days.

The comparative effectiveness of the various triazenes and various standard agents against leukemias 82, 5471, L1210S and L1210AD is demonstrated in Table I.

It can be seen that Triazene III was somewhat more active against leukemia 82 than Triazenes I and IV and that the diethyl triazene (II), was not as effective.

The triazenes were without therapeutic activity against L1210S and L1210AD. In other experiments using variants of leukemia 82 made resistant to amicetin(7), azaserine (8) or aminonucleoside(7), Triazene I was still effective in prolonging survival time.

Using a technique previously reported(9), massive single doses of Triazene I of 1000, 2000, 4000, and 8000 mg/kg of body weight (5, 10, 20 and 40 times the acute LD_{50} dose respectively) were injected intraperitoneally into mice with far advanced 82 leukemia. Bioassay of spleens from these animals into young mice of the same strain 1-2 hours after injection produced leukemia at all levels. This result is in contrast to the sterilizing effects in the same experiment of single injections of 2 to 10 times the LD_{50} dose of one of the more potent nitrogen mustards 1, 2, 3, 4, tetrakis (bis [2-chloroethyl] amino) butane tetrahydrochloride(9,10) and in contrast to the previously reported sterilizing effect of triethylene melamine (TEM) and other alkylating agents on various strains of mouse leukemia.

Discussion. It has been postulated that the chemotherapeutic activity of triazenes is due to their action as alkylating agents. In this respect it should be noted that they are active on strains of transplantable mouse leukemia (82 and 5471) which are also inhibited by the nitrogen mustard methyl-bis (β-chloroethyl) amine (HN2) but that although Triazene I prevents the transplantability of Sarcoma 180 growing on the chorioallantoic membrane of the chick embryo(2) no sterilizing effect could be demonstrated in mouse leukemia in contrast to the activity of HN2, TEM, and other alkylating agents in this respect. Thus it is evident that certain differences as well as similarities exist between the triazenes and the typical alkylating

TABLE I. Comparative Effects of Triazenes on Various Strains of Mouse Leukemia. Mean survival time in days and range.

	Dose, mg/kg	Leukemia 82 Exp. 1	Leukemia 82 Exp. 2	Leukemia 82 Exp. 3	Leukemia 82 Exp. 4	Leukemia 5471	Leukemia L1210S	Leukemia L1210AD
Controls	—	11.2 (11–12)	14.9 (12–17)	14.3 (12–19)	11.8 (10–14)	15.2 (14–17)	13.3 (12–14)	15.4 (8–20)
Triazene I	25	27.8 (22–38)	31.4† (33–50)	30.4 (24–43)	24.1 (19–29)	32.3 (24–49)	13.9 (12–15)	16.4 (13–20)
	12.5	34.2* (22–50)	37.2* (30–50)	42.6‡ (29–50)	39.7 (33–45)	—	—	15.7 (9–20)
II	150	15.5 (12–18)	—	—	—	—	—	—
III	250	34.4* (19–50)	—	—	43.2 (33–62)	—	14.2 (14–15)	14.8 (9–20)
IV	62.5	21.5 (15–28)	—	—	—	—	—	—
	40	—	35.6 (27–44)	—	—	—	—	—
HN2	1	19.0 (12–22)	—	24.6 (22–28)	—	—	—	13.9 (11–17)
Urethane	1000	—	—	22.3 (20–27)	27.3 (20–34)	22.7 (19–28)	—	—
A-Methopterin	3	—	—	—	—	45.7 (35–84)	26.1 (21–37)	13.5 (11–16)

* 1 living at 50 days. † 2 living at 50 days. ‡ 3 living at 50 days.

agents.

In comparative studies on triazene derivatives, addition of the nitro group in the para position of the phenyl group (III) decreased markedly the toxicity of the compound for the host mouse and increased somewhat its relative chemotherapeutic effectiveness against leukemias 82. It was not an effective agent however, against the Amethopterin sensitive or Amethopterin resistant variants of leukemia L1210 which were also refractory to the parent compound (I). Lack of effect of this derivative (III) on development of the chick embryo was attributed by Dagg *et al.* (11) to the fact that in the 4-day-old chick embryo in contrast to the mouse, the addition of the nitro group in the para position of the phenyl group increased rather than decreased the overall toxicity of the compound and thus perhaps made it impossible to give large enough doses to demonstrate a teratogenic effect.

The fact that these triazenes were active against Leukemia 82 which is initially refractory to treatment with Amethopterin and also against lines of Leukemia 82 made resistant to Azaserine, Amicetin, or Aminonucleoside indicates that these compounds are acting through a different mechanism in producing their chemotherapeutic effects.

The above mentioned studies of Clarke *et al.*(1) of Dagg, Karnofsky, *et al.*(2) and the presently reported studies on mouse leukemia suggest that this new series of compounds be given clinical evaluation against neoplastic disease. Such studies are now in progress.

Summary. 1-phenyl 3, 3-dimethyl triazene, has been demonstrated to prolong survival time of mice with transplanted Leukemias 82 and 5471 but not with an Amethopterin sensitive or an Amethopterin resistant variant of Leukemia L1210. A closely allied derivative, 1 - p - nitrophenyl - 3,3-dimethyl triazene has shown relatively greater chemotherapeutic activity against Leukemia 82 but was inactive against the two variants of Leukemia L1210. These compounds were also active against strains of Leukemia 82 which were either initially refractory to or made resistant to Amethopterin, Azaserine, Amicetin, and Aminonucleoside. Further therapeutic studies on this class of compounds are indicated.

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Effect of Adrenalin on Resistance of Adrenalectomized Rats to Certain Toxic Agents. (22274)

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Prior to 1917, the secretion of the adrenal medulla was believed to be the entity which rendered the adrenal glands indispensable for maintenance of life(1). Following the demonstration that epinephrine-free cortical extracts maintained normal blood sugar levels in adrenalectomized rats(2), the role of the medullary hormone has generally been regarded as of minor importance, if any. In searching for a rapid method of assay for DOC-like activity of steroids, it was observed that adrenalin rather than cortical steroids conferred resistance to administration of certain toxic agents in adrenalectomized rats. The sequence of events which led to this finding is here described. On the premise that adrenalectomized animals exhibit decreased tolerance to administered potassium(3), the proposed method of assay was to determine the amounts of cortical steroids which would provide increased resistance to the injection of potassium chloride.

Methods. Intact and adrenalectomized male albino Sprague-Dawley rats, one-half of each group having been treated with desoxycorticosterone acetate, 1 mg rat/day up to 7 days, were injected subcutaneously with 0.5 cc of 10% potassium chloride solution every 10 minutes until death, and the number of injections given up to the time of death was recorded. It was noted that adrenalectomized animals succumbed after only one-half as many injections as those given intact animals, and that even intensive pretreatment with DCA did not diminish the susceptibility of adrenalectomized rats to injected potassium chloride.

Results. Since, in some of the adrenalectomized rats, convulsions suggestive of hypoglycemic shock were noted after only one or 2 injections of KCl, glucose determinations were made on blood collected from the heart at the instant of respiratory failure. These values are shown in Table I.

TABLE I. Blood Sugar of Intact and Adrenalectomized Rats Injected with KCl.

Blood sugar (mg %)*	Untreated	KCl-treated
Intact	135	275
Adrenalectomized	85	45

* Pool of 4 animals.

The blood sugar values confirmed the impression that adrenalectomized rats were indeed in a hypoglycemic state, and also showed that intact animals exhibited an increase in