Brit. J. Pharmacol. (1953), **8**, 278.

OBSERVATIONS ON THE EFFECT OF TRIETHYLENE MELAMINE, AMINOPTERIN, AND A-METHOPTERIN ON THE GROWTH OF TRANSPLANTABLE AVIAN LYMPHOID TUMOURS

BY

C. LE Q. DARCEL

From the Animal Health Trust Poultry Research Station, Houghton, Huntingdon

(RECEIVED OCTOBER 6, 1952)

In recent years numerous substances have been found capable of arresting the development of human leukaemia and certain solid tumours. In view of the practical importance of the avian leukosis complex (Darcel, Lancaster, and Gordon, 1952), it is surprising that the possible application of these drugs in its control has only received scant attention.

The purpose of the present paper is to present observations on the effect of aminopterin, A-methopterin, and triethylene melamine (T.E.M.) on the growth of transplantable lymphoid tumour strains isolated from cases of avian leukosis. Many workers have reported on the favourable effects of these drugs on the course of leukaemic and other neoplastic conditions in man and experimental animals. The first two are typical of the group known as folic acid antagonists, which it appears prevents the conversion of folic acid to the citrovorum factor, essential for nucleic acid synthesis (Nichol and Welch, 1951). It is possible that this interference with nucleic acid metabolism may partially explain the anti-leukaemic action of these drugs (Skipper, Bennett, and Law, 1952). Triethylene melamine is one of a series of tumour inhibitory agents developed by Rose, Hendry, and Walpole (1950). These resemble each other, and active nitrogen mustards, in that each molecule contains at least two alkylating groups. They produce chromosome fragmentation and bridge formation in proliferating cells. The cytotoxic action of this group might also partially be attributed to a specific inactivation of enzymes (Haddow, 1947).

The general characteristics of the tumour strains used in the present study, RPL 12, 16, and 19, have been described by Olson (1941), Burmester and Prickett (1945), and Burmester (1947). In experiments described by Darcel (1952), these three tumours killed 90–100% of chicks, inoculated subcutaneously, in 8–10 days. A tumour develops at the site of inoculation followed by rapid metastasis, in the absence of leukaemia, to the viscera, especially the liver.

METHODS

Crossbred (B.R. × Br. Leghorn) chicks were inoculated with tumour cell suspensions in the first few days of life. The chicks were maintained in small metal cloches and were fed *ad lib.* on a mash based on national standards.

The tumour cell suspensions were prepared in physiological saline with a special mincer (Craigie, 1949) from liver or pectoral muscle in which tumours were growing; they were filtered, before inoculation, through butter muslin and sintered glass filters (AG74 × 0) so that the tumour cells were more evenly distributed. The tumour suspension (0.25 ml.) was inoculated subcutaneously above the pectoral muscle. Concentrations of the tumour mince used corresponded to 1/10th of the original tumour in all experiments. Further dilutions (1/100, 1/1,000, and 1/10,000) were used in the studies on the effect of oral administration of T.E.M. and folic acid antagonists. The chicks were palpated daily for the calculation of mean time for first appearance of tumours in individual groups. In the experiments with aminopterin and A-methopterin the chicks were kept for three weeks only, but in the experiments with T.E.M. the observation period was extended to four weeks so that the mean survival times of the experimental groups could also be determined.

RESULTS

Triethylene Melamine (T.E.M.).—Rose, Hendry, and Walpole (1950) showed that T.E.M., given orally to rats, would inhibit tumour growth. This mode of administration in chicks is so convenient that it was used in three separate trials in which

the drug was given in the drinking water in concentrations up to 0.01% after inoculation. It had no significant effect on the length of time before tumours appeared or on the time that chicks survived after tumour transplantation. Although in this series of experiments no inhibitory effects of T.E.M. on the growth of these lymphoid tumours were demonstrated, there was evidence of a toxic effect in that chicks treated with the drug gained less weight. In the experiment in which 0.01% T.E.M. was administered in the drinking water, six of the twelve chicks not inoculated with tumour material died, the survivors weighing only 105.67± 14.9 g. in comparison with 151±5.25 g. for untreated. Corresponding mean weights for the surviving chicks which had been inoculated with tumour material were 89.38±5.58 g. and 143.67± 8.27 g. for the treated and untreated groups respectively. Chicks given T.E.M. at this dose level would have each received approximately 43, 96, and 160 mg. of the drug by the end of the second and third weeks respectively.

A different picture was seen when the drug was administered by the intraperitoneal route (Table I). In experiments 1 and 2 administration of the drug was delayed until palpable tumours appeared, whilst in experiments 3 and 4 administration was begun the day after implantation of tumour cells. In experiment 1 each chick received three daily intraperitoneal injections of a 0.001% solution of T.E.M. in distilled water. In experiment 2 four injections were given in six days and in experiments 3 and 4 five injections in 10 days.

TABLE I

THE EFFECT OF INTRAPERITONEAL ADMINISTRATION OF TRIETHYLENE MELAMINE ON THE GROWTH OF TRANSPLANTABLE AVIAN LYMPHOID TUMOURS

Exp. No.	Total Amount of T.E.M. Administered	Chicks Inoculated	Tumours		Deaths	
			Tumours Palpated	Mean Time for First Appearance in Days	No. of Deaths	Survival Time in Days
1	1·5 mg.	10	10	4·60±0·16	10	9·90±0·28
	0·75 „	10	10	4·80±0·13	10	12·00±0·75
	0	9	9	4·67±0·15	9	7·44±0·18
2	0·25 „	10	10	9·10±0·10	7	13·57±0·43
	0	10	10	9·20±0·13	9	12·22±0·33
3	0·31 „	10	9	8·56±0·33	8*	12·50±0·57
	0	10	10	6·10±0·10	10	9·30±0·15
4	0·31 „	10	10	5·40±0·34	10†	12·10±0·85
	0	10	10	4·10±0·10	10	6·90±0·18

* Two were killed with tumours 17 days after inoculation. One showed a very large ulcerated pectoral tumour. In both cases there was tumour involvement of the proventriculus.

† One died at 10 days from causes apparently not associated with tumour growth.

In experiments 1 and 2 tumour strain RPL 19 was used, and in experiments 3 and 4 RPL 12.

Other chicks were treated with T.E.M. in the same way, but were not implanted with tumour cells. Those which received 1.5 mg. died after 9.90±0.18 days and those which were given 0.75 mg. died after 12.00±0.78 days. Of the others, which received 0.31 or 0.25 mg., only two out of thirty died within the experimental period. It will be seen from Table I that in spite of these toxic effects of T.E.M. the chicks implanted with tumours and given T.E.M. lived longer than untreated chicks. In experiment 1 the untreated controls survived only 7.44 days, those which received 0.75 mg. 12.00 days; 1.5 mg. T.E.M. reduced the survival time to 9.90 days because of its own toxic effects. In experiment 2 the dose of T.E.M. was less and tumour implanted chicks survived only a day longer than the untreated controls. In experiments 3 and 4, in which the drug was given before tumours appeared, there was a significant increase both in the time which elapsed before tumours appeared and in the survival time.

Aminopterin and A-methopterin.—In preliminary experiments chicks which were given five daily doses of aminopterin and A-methopterin in aqueous suspension (0.1 and 1.0 mg. of each drug per day), administered directly into the crop, showed a significant gain in weight compared with untreated controls. Only one out of 29 chicks died in this series.

The effect of the administration of aminopterin and A-methopterin on the growth of RPL 12 was tested by implanting groups of ten chicks with concentrations of tumour suspensions equivalent to 10^{-1}–10^{-4} of the original undiluted tumour mince. Five chicks in each group were given 0.01 mg. of the drug daily. The times during which tumours developed varied from 6–7 days from the chicks receiving the 10^{-1} suspension to 10–11 days for chicks receiving the 10^{-4} suspension. No significant differences were observed between the treated and untreated groups in the time for appearance of tumours.

DISCUSSION

In T.E.M. treated chicks in which the drug was given parenterally before the appearance of tumours, the latter took longer to become apparent, as judged by palpation, and there was an increased survival time. In the detection of tumours there is naturally a subjective error, but this is surprisingly small for these rapidly growing tumours (Darcel, 1952). The absence of any significant effect on tumour growth when the drug was given by the oral route is difficult to explain. The marked reduction in growth rate following treatment, both in tumour inoculated and uninoculated

chicks, suggests that absorption of the drug had occurred. This drug is not particularly stable, and it is possible that through the presence of reactive contaminants in the drinking water and intestine it might have been altered to an inactive but still toxic form.

No increase in the time taken for tumours to appear in treated chicks was observed in the experiments with aminopterin and A-methopterin. However, since there was no evidence of toxic effects when these drugs were given orally, and in view of the finding by Little *et al.* (1945) that a few doses of 10 micrograms given intraperitoneally to chicks were highly toxic, it must be assumed either that there is very little absorption in the intestinal tract or that an alteration of the drugs takes place. The fact that a significant weight increase occurred in chicks receiving aminopterin suggests that it is the latter explanation that is correct. Under these circumstances it is not surprising that aminopterin and A-methopterin given orally failed to influence tumour growth. In this connection it is of interest that the growth of the Rous sarcoma is unaffected by the administration of aminopterin in the food (Ringsted, 1952), but inhibited when this drug is given parenterally (Little *et al.*, 1945).

The absence of an effect of T.E.M. on the survival time when the drug was given parenterally after palpable tumours appeared recalls the experiments of Geisse and Kirschbaum (1950) with urethane and sodium arsenite on the course of mouse leukaemia. The maximum effect was obtained only if these were given shortly after transplantation of the leukaemia.

The inhibitory effect of parenteral administration of T.E.M. on the growth of these lymphoid tumours suggests that this compound might be given field trials in attempts to prevent the development of spontaneous lymphomatosis. Such attempts should be postponed until more is known about the toxicity and possible carcinogenic activity of this compound.

Summary

1. Triethylene melamine given orally had no significant effect on the growth of transplantable avian lymphoid tumours. Given parenterally before the appearance of palpable tumours, the drug had some inhibitory effect on tumour growth but was highly toxic. If treatment was delayed until the tumour appeared there was no significant effect on the survival time of inoculated chicks.

2. Aminopterin and A-methopterin given orally to chicks bearing tumour strain RPL12 had no significant effect on growth. The absence of toxic effects following much heavier dosage suggests that the chicks did not absorb the drug in sufficient quantity.

These studies have been made with the aid of a grant from the Agricultural Research Council. Dr. A. L. Walpole, of the Imperial Chemical Industries Ltd., kindly provided the triethylene melamine, whilst the aminopterin and A-methopterin were a gift from the Lederle Laboratories Division of the American Cyanamid Co.

References

Burmester, B. R. (1947). *Cancer Res.*, **7**, 786.
—— and Prickett, C. O. (1945). *Poult. Sci.*, **5**, 652.
Craigie, J. (1949). *Brit. J. Cancer*, **3**, 249.
Darcel, C. le Q. (1952). Ph.D. thesis, University of London.
—— Lancaster, J. E., and Gordon, R. F. (1952). *Brit. vet. J.*, **9**, 338.
Geisse, N. C., and Kirschbaum, A. (1950). *Cancer Res.*, **10**, 108.
Haddow, A. (1947). *Brit. med. Bull.*, **4**, 417.
Olson, C., Jr. (1941). *Cancer Res.*, **1**, 384.
Little, P. A., Sampath, A., Paganelli, V., Locke, E., and Subbarrow, Y. (1945). *Trans. N.Y. Acad. Sci.*, **10**, 91.
Nichol, C. A., and Welch, A. D. (1951). *Proc. Soc. exp. Biol., N.Y.*, **74**, 403.
Ringsted, J. (1952). *Acta path. microbiol. scand.*, **31**, 275.
Rose, F. L., Hendry, J. A., and Walpole, A. L. (1950). *Nature, Lond.*, **165**, 993.
Skipper, H. E., Bennett, L. C., Jr., and Law, L. W. (1952). *Cancer Res.*, **12**, 677.