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Influence of the tissue distribution of ThioTEPA and its metabolite, TEPA, on the response of murine colon tumours

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Summary. Disposition studies in the same animals as those used for assessment of antitumour and toxic effects could increase understanding of the variation in response to cytotoxic drugs. Tissue and plasma levels of ThioTEPA and triethylenephosphoramide (TEPA) were measured to see if any correlation existed between them and the effects of the drug on a series of mouse colon tumours (MAC). The tumour panel included an ascitic form (MAC 15A), an anaplastic (MAC 13) and a well-differentiated (MAC 26) solid tumour, all grown subcutaneously.

The maximum tolerated dose of ThioTEPA was 20 mg kg^{-1} in females bearing MAC 13 and 15 mg kg^{-1} in males having MAC 15A or 26. The diverse growth characteristics of the tumour cell lines necessitated the use of different methods for assessment of response. After administration of the maximum tolerated dose, the greatest response was observed in MAC 26, in which a growth delay of 15 days – twice the doubling time of the tumour volume – occurred. ThioTEPA produced 58% inhibition of MAC 13 tumour weight, but MAC 15A was unresponsive.

One hour after intraperitoneal administration of ThioTEPA (20 mg kg^{-1}), ratios of tissue to plasma concentration were 1.13, 0.87 and 1.17 in tumours and 0.80, 0.75 and 0.73 in spleens of mice bearing MAC 13, 15A and 26 respectively. These data show greater accumulation of drug in neoplastic than in normal tissues. The pattern of distribution of the metabolite was similar, but there was a lesser degree of tissue accumulation than by the drug. Concentrations of drug and metabolite in neoplastic tissues related to their protein content were 116.0, 126.3 and 183.3 μg ThioTEPA/g and 57.5, 83.1 and 78.6 μg TEPA/g in MAC 13, 15A and 26 respectively.

Combination of these chemosensitivity and pharmacokinetic data indicates that differences in response of these tumours to ThioTEPA cannot be explained by the availability of the drug and metabolite. The therapeutic effects of ThioTEPA cannot be predicted purely from a knowledge of drug and metabolite disposition.

Introduction

The cytotoxic drug, triethylenethiophosphoramide (ThioTEPA) is used widely in instillations for superficial bladder carcinoma [15] and is currently being employed in combination regimes for advanced ovarian and breast cancers [17]. For the purpose of clinical pharmacokinetic studies, a sensitive and specific assay was developed [10] to determine concentrations of ThioTEPA and its major metabolite, triethylenephosphoramide (TEPA), which also has significant antineoplastic activity. As an adjunct to patient investigations of the biotransformation and excretion of ThioTEPA in relation to its toxic side effects, its disposition and also the distribution of drug and metabolite was studied in normal mice in order to gain insight into the mechanism of elimination [11]. At a therapeutic dose (20 mg kg^{-1}) peak plasma levels of both ThioTEPA and TEPA occured 1 h following intraperitoneal (i.p.) treatment.

A case has recently been made for detailed pharmacokinetic studies to be caried out in the same tumour-bearing animals used to assess the therapeutic efficacy of cytotoxic agents [18]. Based on such studies a prediction may be made of the minimum concentration and time of exposure required for antitumour activity in the patient.

The mouse adenocarcinoma of the colon (MAC) series of transplantable tumours in NMRI mice [4] has been used in a variety of chemotherapy studies, and two of these tumours have been shown to be sensitive to ThioTEPA [6]. The main objectives of this study were to determine the concentrations of ThioTEPA and TEPA in a panel of MAC tumour lines of different histology and with different growth characteristics and to correlate tumour levels at the time of peak plasma levels with chemotherapeutic response.

Materials and methods

Animals. Pure-strain NMRI mice (aged 6–8 weeks) from our inbred colony were used. They were fed on CRM diet (Labsure, UK) and water ad libitum.

Materials. Spectroscopic-grade ethanol (BDH Chemicals, Poole, Dorset, UK), hexaethylphosphoramide (Fluorchem Ltd., Glossop, Derbyshire, UK), bovine serum albumin (Sigma Chemical Co., Poole, Dorset, UK), and triple-distilled water were used. Other reagents were of analytical grade. ThioTEPA was a gift from Lederle Laboratories, Gosport, Hants, UK.

Tumour system. The series of transplantable murine colon tumours used in this study were developed from primary

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tumours induced by dimethylhydrazine [8]. These adenocarcinomata are very similar in terms of histology, cell kinetics and chemosensitivity to tumours of the human colon [3] and range from very well differentiated mucin-producing tumours to anaplastic, ascitic tumours. The tumours used for this study were MAC 13, MAC 15A and MAC 26. MAC 13 was implanted into female mice; MAC 15A and MAC 26 were implanted into male mice.

Chemotherapy. The differing morphology and growth characteristics of the tumour lines employed necessitated the use of different chemotherapy and assessment protocols as described previously [5]. ThioTEPA was dissolved in sterile 0.9% saline and injected i.p.

Preparation of tissue and plasma specimens for distribution studies. A minimum of five mice from each tumour cell line were treated with a single i.p. dose of ThioTEPA (20 mg kg^{-1}). In control experiments, tumour-bearing mice were given a similar i.p. dose of physiological saline. One hour after administration animals were anaesthetised with diethyl ether, and blood samples obtained by cardiac puncture were collected in heparin tubes on ice. The mice were then killed by cervical dislocation and rapidly dissected. Tumours and spleens were immediately frozen and stored in liquid nitrogen until extracted for analysis. Spleens were used as normal control tissues. Blood samples were centrifuged at 2000 g and 4° C for 10 min, and plasma was frozen and stored at −30° C. Tissues and cells were homogenised in distilled water (10%, w/v) using an Ultra Turrax blender. Homogenates were centrifuged at 2500 g and 4° C for 15 min; the volume of the supernatant was noted and was extracted for analysis.

Analysis of drug and metabolite. After extraction of ThioTEPA and TEPA from biological samples using solid-phase chromatography, the compounds were separated by capillary gas chromatography (GC), detected using a nitrogen detector and quantified by reference to an internal standard, hexaethylphosphoramide [10]. The limit of sensitivity was 1–5 ng/ml. At a concentration of 500 ng/ml, which was within the range found in these samples prepared for extraction, analytical recovery of ThioTEPA was 97.5%, and intra- and interassay variations (SE) were 5.5% and 8.0% ($n = 6$) respectively. Recovery of TEPA was 75.2% and assay variations were 5.7% and 7.0%; however, because of the lack of chemical stability and difficulty of preparation of authentic TEPA, the metabolite was determined as ThioTEPA eqivalents in this study.

Protein estimations. Determination of the protein content of homogenised tissue (250:1) was carried out in duplicate [9]. Bovine serum albumin was used as a standard over the range 0–200 μg/ml.

Statistical analysis. Statistical analysis was performed using the Student's *t*-test to examine significant differences within the groups.

Results

Chemotherapy

The maximum tolerated dose of ThioTEPA in female tumour-bearing mice was 20 mg kg^{-1}, whereas this dose was only 15 mg kg^{-1} in male mice bearing MAC 26 tumours. The response of MAC 13 tumours to ThioTEPA is described in Table 1. The anti-tumour activity was assessed by examining the ratio of tumour weight of treated animals and controls. At maximum tolerated dose a T/C value of 42% was achieved, and this represents a 58% inhibition of tumour weight. The chemosensitivity of MAC 15A cells implanted s.c. was assessed by the same technique (Table 1). There was no anti-tumour activity at maximum tolerated dose.

The activity of ThioTEPA against MAC 26 tumours was determined by assessing growth delay at a relative tumour volume of 5. This tumour has a volume-doubling time of approximately 7 days. A dose level of 15 mg kg^{-1} produced a growth delay of 15 days (Table 1) and 20 mg kg^{-1} produced a delay of 38 days at the expense of host toxicity.

Distribution studies of drug and metabolite

In control experiments, no interfering components were found in plasma, tissues or peritoneal fluid which would affect the accuracy of the analyses of drug and metabolite.

ThioTEPA concentrations in plasma and tissues of tumour-bearing mice are given in Table 2. Levels of the drug in the MAC 13 and 26 tumours were significantly ($P > 0.05$) greater than in the spleens of these tumour-bearing animals. In the mice with the MAC 15A cell line, however, ThioTEPA concentrations in tumour and spleen tissue were similar.

A similar trend in the results of analyses of TEPA was obtained (Table 3), in which concentrations were significantly ($P < 0.05$) greater in the MAC 13 and 26 tumours than in the corresponding spleens; there was no significant difference in tissue levels in mice bearing the s.c. MAC 15A tumour.

Expression of the concentrations in terms of protein content does not alter the conclusions.

Table 1. Anti-tumour effects of ThioTEPA

Tumour	Dose (mg kg^{-1})	Survivors/10	Percentage inhibition	Growth delay (days)	T/C %
MAC 13	25	6		–	–
	20	10	58	–	42
	15	10	16	–	84
MAC 15A s.c.	20	10	0	–	100
MAC 26	25	5	–	–	–
	20	9	98	38	2
	15	10	90	15	10

Table 2. Concentrations of ThioTEPA in plasma and tissues of tumour-bearing mice 1 h after i.p. injection of ThioTEPA (20 mg kg^{-1})

Tumour line	Mean concentration of ThioTEPA ± SE		
	Spleen (μg g^{-1})	Tumour (μg g^{-1})	Plasma (μg ml^{-1})
MAC 13	3.90 ± 0.12	5.52 ± 0.40	4.88 ± 0.89
MAC 15A s.c.	5.73 ± 0.81	6.61 ± 0.93	7.60 ± 0.99
MAC 26	4.05 ± 0.23	6.45 ± 0.26	5.52 ± 0.13

Table 3. Concentrations of TEPA in plasma and tissues of tumour-bearing mice 1 h after i.p. injection of ThioTEPA (20 mg kg^{-1})

Tumour line	Mean concentration of TEPA (as ThioTEPA equivalents) ± SE		
	Spleen ($\mu g\ g^{-1}$)	Tumour ($\mu g\ g^{-1}$)	Plasma ($\mu g\ ml^{-1}$)
MAC 13	1.93 ± 0.17	2.71 ± 0.25	5.49 ± 1.24
MAC 15A s.c.	4.59 ± 0.59	4.33 ± 0.40	5.86 ± 0.45
MAC 26	1.91 ± 0.14	2.79 ± 0.35	3.99 ± 0.11

Discussion

Initially TEPA, and then ThioTEPA was introduced into clinical use approximately 30 years ago, at a time when pharmacokinetic studies were not the major contribution to an understanding of antitumour activity as they are today, and the majority of investigations addressed only one of these aspects.

In early studies TEPA was shown to exert remarkable inhibitory effects on the growth of some experimental rat tumours, including the R 39 and Jensen's sarcoma, but decreased responses were observed in the Flexner-Jobling carcinoma and Walker 256 carcinosarcoma and the agent had no effect on the Murphy-Sturm lymphosarcoma [2, 16]. Similarly, we have observed a range of responses to ThioTEPA in the panel of MAC tumours investigated. The maximum tolerated dose of the drug had the greatest effect on the proliferation of the MAC 26 tumour, in which a growth delay of twice the volume-doubling time occurred (Table 1). A lesser response, 58% inhibition of tumour weight, was obtained in the MAC 13 cell line, whereas MAC 15A was unresponsive.

The specific detection each of ThioTEPA and TEPA simulteneously in tissue specimens has not been performed previously. The majority of studies of the distribution of ThioTEPA or TEPA have utilized the radiolabelled compounds. Nadkarni et al. [12] showed that after administration of [^{32}P]-TEPA, accumulation of radioactivity occurred in Sarcoma 37 and Lymphosarcoma 1 in CAF_1 mice but not in the Leukaemia L1210 in the DBA/2 strain. There was no marked selective localisation of radioactivity in the sarcomas in comparison with control tissues including the spleen. Also utilizing [^{32}P]-TEPA, Craig and Jackson [2] reported that radioactivity accumulated in the kidney, liver and spleen but not in the Jensen sarcoma in rats. No specific localisation of radiolabelled material occurred in tissues of the mouse after dosage with [^{32}P]-ThioTEPA [1]. In rats bearing the Walker 256 carcinosarcoma and given [^{14}C]-ThioTEPA, levels of the unaltered drug were similar in tumour and spleen tissues, but there was a striking accumulation of total radioactivity in the tissues of the gastrointestinal tract, the majority of which was present as metabolized drug [14]. Similarly, we have found from distribution studies in normal mice that 1 h after administration of ThioTEPA the highest level of the drug is found in the caecum, and TEPA is most concentrated in the small intestine (B. J. McDermott et al. unpublished data). Using GC methodology for specific determination of ThioTEPA, Egorin et al. [7] have more recently investigated drug distribution in normal tissues of the mouse. One hour after administration of 5 mg kg^{-1}, ThioTEPA was not detectable on any of the samples, and at times up to 30 min the concentration in spleen was approximately 33% of that in plasma. Using four times this dosage, the level necessary to achieve responses, we have found that in the MAC tumour-bearing mice the ThioTEPA levels in spleens were a greater proportion of the corresponding plasma concentrations (mean 72%, calculated from Table 2). Drug levels in the MAC tumours were similar (Table 2), and these findings were reflected in the corresponding TEPA concentrations (Table 3).

The equilibrium distribution ratio of concentration in tissue to concentration in blood indicates the extent of penetration of a substance into a particular tissue [13]. Using plasma data to calculate such indices does not provide a valid estimate if the material is sequestered in cellular components of the blood. This is the case with ThioTEPA, as has been shown by the study of Craig et al. [1], in which concentrations of radioactivity in murine blood were approximately twice those in plasma over a period of 3 h after administration of labelled drug. The results from this study do show, however, that ThioTEPA accumulates in tissues more easily than does TEPA, which is consistent with the greater polarity of the metabolite. This was demonstrated previously by the inhibition of metabolite but not of drug clearance by concomitant i.p. administration of arachis oil [11].

The anti-tumour data indicate no correlation between chemotherapeutic response and tissue distribution of ThioTEPA and TEPA. The methodology described here does not allow for measurement of alkylation of tissue macromolecules but does demonstrate that bioavailability is similar for the three tumour lines described.

In conclusion, it is clear that tumour disposition studies with ThioTEPA alone are insufficient to predict chemotherapeutic responses of experimental mouse colon tumours, and measurement of drug levels must be carried out in conjunction with anti-tumour assessments. These studies are being extended to investigate whether in vitro cytotoxicity assays in conjunction with pharmacokinetic and metabolism studies can be used to predict in vivo responses of transplantable tumours to ThioTEPA.

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