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TUMOUR SULPHYDRYL LEVELS AND SENSITIVITY TO THE NITROGEN MUSTARD MEROPHAN*

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Abstract—It has been argued that the intrinsic sulphydryl levels of tumours should affect their response to alkylating agents. This has been tested experimentally using six different mouse tumours and Merophan as a chemotherapeutic agent. No correlation between tumour sensitivity and protein bound or acid soluble SH levels was found. A good correlation between tumour sensitivity and the ratio of protein bound : soluble SH was obtained. Further implications of this finding are discussed.

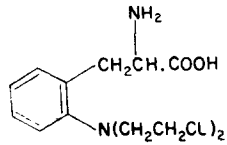
ONE of the problems associated with the chemotherapy of tumours is the wide range of sensitivities of different tumours to treatment with any one agent. A similar situation applies in the case of transplantable animal tumours where an alkylating agent may cause complete regression of one tumour and have no effect whatsoever on another. These differences in behaviour could arise from anatomical considerations or from inherent biochemical differences in tumours.

A variety of mouse tumours have been examined with regard to their response to an alkylating agent and in relation to their intrinsic sulphydryl SH levels. The choice of SH levels as a biochemical parameter has been determined by a number of factors. Alkylating agents are known thiol reactants and *in vivo* some are metabolized (in part) by conjugation to SH containing tissue components.

It has been found by Calcutt and Doxey[2] that nitrogen mustard, Chlorambucil and Myleran will cause well defined falls in the levels of both protein bound and trichloroacetic acid (TCA) soluble levels of mouse skin.

Many workers have shown that thiol containing compounds are effective protective agents against the toxicity of the alkylating agents.[3–6] Recent work by Calcutt, Connors, Elson and Ross[7] has demonstrated that the extent of protection conferred by cysteine is directly associated with the level of acid soluble SH in tissues sensitive to the alkylating agent. In this event the level of acid soluble SH normally present in a tumour should be expected to influence the susceptibility of that tumour to treatment with an alkylating agent.

* *o*-Di-2-chloroethylamino-DL-phenylalanine.[1]



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We have measured the intrinsic sulphydryl levels of six types of experimental mouse tumours and correlated the results with the inhibition of their growth by Merophan at the maximum tolerated dose.

EXPERIMENTAL

Details of the tumours are given in Table 1. The Merophan has been given by intraperitoneal injection at the maximum tolerated dose level as determined on tumour bearing animals. We have also determined the total free SH, TCA soluble SH, and by difference of these two figures, the protein bound SH for tumours taken at the same time as those treated with Merophan. All SH measurements have been made by the methods described by Calcutt and Doxey[8] and Calcutt, Doxey and Coates.[9]

TABLE 1. DETAILS OF EXPERIMENTAL TUMOURS

Mouse strain	Tumour	Tumour type
Strong A	M.V. 285	Lymphoma
Balb/C+	Spontaneous	Mammary carcinoma
Mixed stock	180	Sarcoma
Balb/C−	Harding Passey	Melanoma
C57/Dba2	755	Mammary carcinoma
Balb/C−	ADJ/TC5	Myeloma

All transplanted tumours were grown as subcutaneous grafts in the flanks.

RESULTS

The basic findings are given in Table 2. The estimates of inhibition of the Merophan treated tumours are derived from the relative weights of the treated and untreated tumours about 12 days after injection of the Merophan in a number of test runs using ten to twenty mice per group. Because of the requirement for adequate comparable tissue for SH measurements all tumours have weighed approximately 0·5 gm at the time of treatment.

The number of animals used for SH measurements are indicated in Table 2. In some cases the TCA soluble SH value exceeds the total SH value for the same tumour. This apparently anomalous finding has also been regularly encountered in work with foetal tissues, rat spleen and hamster kidneys. Because of its predictable occurrence under defined conditions we considered this to be a reflection of the biochemistry of the tissue concerned and not a fault of the techniques used.

Inspection of the figures in Table 2 shows that there is little or no relationship between the extent of inhibition of these tumours and the overall SH values. When the mean value of the ratio of protein bound SH to TCA soluble SH for each tumour is plotted against the percentage inhibition as in Fig. 1 a very good correlation is obtained.

The results have been examined in an alternate fashion. For each tumour a frequency distribution plot of the ratios of protein bound to soluble SH obtained with individual samples has been drawn. Then each plot has been divided by a vertical line drawn so that the number of points falling to the right of it (i.e. in the region of higher ratios) is an approximation to the inhibition which could have been expected

TABLE 2.

Tumour	Chemotherapy				—SH measurements			
	Time for establishment (days)	Period of test (days)	% inhibition	Numbers of animals	Total —SH	TCA soluble —SH	Protein Protein —SH	Protein bound:soluble ratio
Carcinoma 755	15	11	50	15	7·6 ± 1·6	4·4 ± 0·7	3·2 ± 1·7	0·8 ± 0·45
Mammary carcinoma	—	12	0	15	6·4 ± 1·2	5·4 ± 0·7	1·0 ± 1·5	0·2 ± 0·3
Sarcoma 180	4	9	30	18	5·0 ± 0·7	3·7 ± 0·7	1·2 ± 1·1	0·4 ± 0·4
Harding–Passey melanoma	24	12	0	14	9·5 ± 2·6	8·2 ± 1·9	0·75 ± 3·1	0·2 ± 0·3
Myeloma	10	9	48	16	7·2 ± 0·8	3·9 ± 0·6	3·3 ± 0·8	0·9 ± 0·3
Lymphoma	10	14	100	36	10·65 ± 2·1	3·6 ± 0·7	7·1 ± 2·0	2·0 ± 0·8

All —SH measurements are expressed as μg —SH/100 mgm wet weight of tissue.

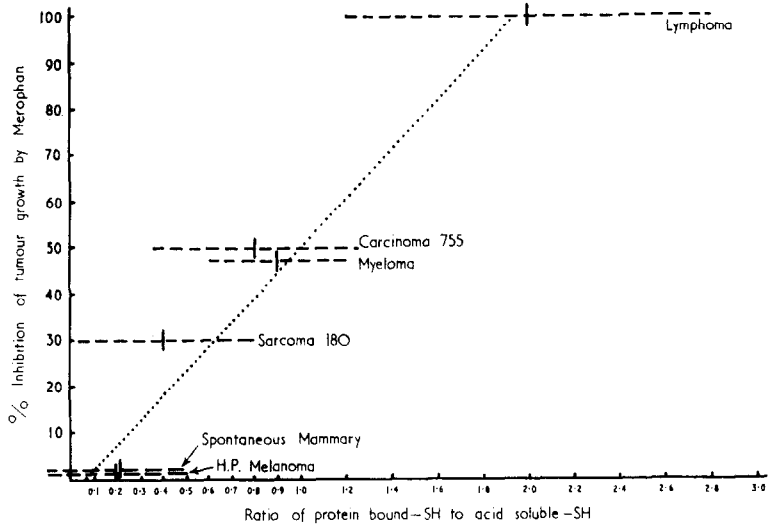

FIG. 1. The relationship of percentage inhibition of tumour growth and the protein-bound to soluble —SH ratio of six mouse tumours. The dash line indicates the extent of the standard deviation of the ratio measurements. The dotted line is the best straight line fit to the experimental points.

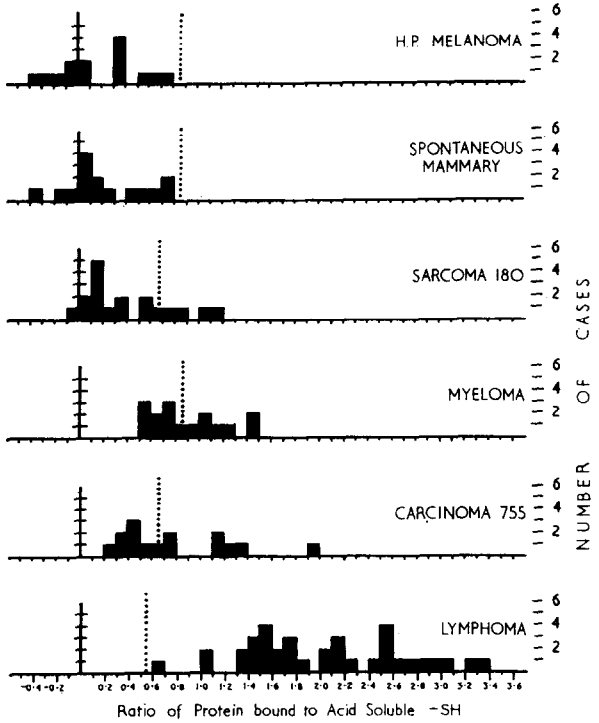

FIG. 2. The frequency distribution of protein-bound to soluble —SH ratios for different tumours.

if the tumours used in these measurements had been subjected to a maximum tolerated dose of Merophan. These plots have been collected together as Fig. 2 and it will be seen that the vertical lines fall in a very restricted range. It is concluded from this that if an individual tumour has a protein bound: soluble SH ratio in excess of 0·6–0·8, then that tumour is sensitive to Merophan, provided the dosage requirements are met.

DISCUSSION

Alkylating agents can theoretically react with many cell constituents, and it would be unwise to assume, on the present evidence, that a reaction with one specific component of the cell is responsible exclusively for the anti-tumour action of the alkylating agent. It is known that mustards will alkylate the guanine moiety of nucleic acids,[10] and a probable explanation of the anti-tumour action of the alkylating agents is a primary reaction with DNA. However, there is also good evidence that *in vivo* the major reaction of Myleran (a *bis* sulphonoxyalkane) is an alkylation of cysteine thiol groups,[11] and present techniques could not have detected the more labile linkages which could have formed at other sites (see review by Ross in *Biological Alkylating Agents*[12]).

Deactivation of known thiol containing enzymes would not seem to be an important factor since they are relatively insensitive to alkylating agents at doses required to cause tumour inhibition. Ross has pointed out however,[12] that there may well be an enzyme, as yet unknown, which will prove to be highly sensitive to these agents. Peters[13] also considers that an attack on cell surfaces (e.g. by alkylation of structural thiol groups) with a consequent modification of permeability is a possible mode of action of the alkylating agents.

If alkylation of certain protein bound SH components contributes in part to the anti-tumour action of the alkylating agents, then our preliminary results would indicate that the cellular acid soluble SH is protective to the protein bound SH. The ratio of the two then becomes important for this will help to determine whether an alkylating agent is detoxicated by the acid soluble SH or inflicts damage on the protein bound SH.

This concept of competition between the acid soluble and protein bound fraction of the intracellular SH has only been considered in terms of treatment with Merophan, but it is to be expected that similar considerations will apply in the case of other alkylating agents, as these behave both chemically and biologically very much the same as Merophan. This same concept of competition would also appear to be applicable to considerations of other drugs or foreign compounds which react with sulphydryl groups.

This finding also opens up the possibility of further research aimed at altering the intracellular level of SH in a tumour so as to render it more sensitive to chemotherapy. It has already been shown by Alexander[14] that cells in culture are made more sensitive to X-rays if pretreated with iodoacetate which blocks intracellular SH groups.

A reason for the apparent relative selective action of alkylating agents upon certain tumours is seen in the fact that normal mouse liver, which is not noticeably damaged by most alkylating agents, has a protein bound: soluble SH ratio of $0{\cdot}4 \pm 0{\cdot}3$. At this value the liver would be relatively resistant as compared with many tumours.

The present results also throw light upon the problem of induced resistance in tumours occurring after treatment with chemotherapeutic agents. Such resistance can

now be envisaged as a shift of the intracellular SH ratio towards a lower value. That such a change could occur is a reasonable assumption in view of the known ability of tissues to overcompensate when they have been affected by extraneous agents. Calcutt and his associates (see summary by Calcutt[15]) have shown that carcinogenic agents will cause increases in target tissue SH levels, and in this connection it must be remembered that the agents used in cancer chemotherapy are themselves carcinogens. Recently Hirono[16] found that Yoshida ascites sarcoma and ascites hepatoma cells (AH.13 and AH.7974) which have become resistant to nitrogen mustard have a higher acid soluble SH level than do the normal cells.

Unfortunately no measurement of protein bound SH was made, but a record of total SH measurements was made. So, taking the protein bound as the difference between the total and acid soluble figures as previously, it has been possible to calculate protein bound to soluble SH ratios as before. These figures are given in Table 3

TABLE 3. SENSITIVE AND RESISTANT TUMOURS
DATA DERIVED FROM HIRONO[16]

Tumour	—SH measurements			
	Total —SH	TCA soluble —SH	Protein-bound —SH	Protein-bound:soluble ratio
Yoshida Ascites				
Sarcoma (original)	15·8	2·5	13·3	5·3
Sarcoma (resistant)	14·1	5·7	8·4	1·5
Hepatoma AH 13 (original)	12·4	3·1	9·3	3·0
Hepatoma AH 13 (resistant)	13·6	5·3	8·3	1·55
Hepatoma AH 7974 (original	12·9	4·0	8·9	2·2
Hepatoma AH 7974 (resistant)	14·7	5·5	9·2	1·65

The figures for protein bound —SH and the protein bound:soluble ratio have been calculated from Hirono's original data. —SH measurements are given as μM —SH/gm wet weight of tissue.

and are completely in agreement with the suggestion that resistance is accompanied by a decrease in this ratio. The actual values are of a different order from those obtained in the present work, but the relative changes are as to be expected.

The subject of radiation protection has also become involved with questions of intracellular thiol groups and it appears possible that similar considerations to those expressed above may apply to questions of radiosensitivity and to induced radio resistance.

The work described in this paper is only the early stages of an extensive investigation, but because of the obvious importance of these initial findings it is being published now. It is hoped that this will stimulate other workers having information associated with this material to publish their data.

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