Biochemical Pharmacology, 1966, Vol. 15, pp. 753–754. Pergamon Press Ltd., Printed in Great Britain.

In vitro decomposition rate of degranol in sensitive and resistant NK/lymphoma ascites tumours

(*Received* 17 *September* 1965; *accepted* 13 *January* 1966)

KÖRÖS *et al.*,[1] have studied the rate of decomposition of some alkylating agents in animal and human blood both *in vivo* and *in vitro*. In these experiments the method of Klatt[2] proved to be suitable for demonstrating the presence of Degranol and its decomposition. Obrecht *et al.*[3] by a similar method have followed the rate of decomposition of a mustard derivative HN_2 in various organs of rats.

In previous work we have obtained a strain of the NK/lymphoma ascites tumour with acquired resistance to Degranol.[4] The object of the present work has been to determine whether a difference can be demonstrated between the rate of decomposition of Degranol by sensitive and by resistant tumours.

METHODS

One hundred and fifty-seven resistant tumours from the sixtieth to eightieth passages and 140 sensitive tumours were used. After inoculation of 2×10^6 cells, the animals were killed 8 days later and the ascites drained. Degranol (100γ) per ml was added to the tumour. Determinations were carried out immediately after addition of the Degranol and 1, 2, 3, 4, 6 and 18 hr after incubation, using the method of Klatt.[2] All incubations were at 37°, under sterile conditions and with continual shaking.

For each determination sufficient tumour was taken to give 1 ml of supernatant after centrifugation. To 1 ml of the supernatant was added 3 ml of dilute acetic acid and the pH adjusted to 4·5. After addition of 0·5 ml of 5% 4-γ-nitro-benzylpyridine dissolved in methyl ethylketone, the mixture was shaken and placed in a water bath for 15 min at 80°. After cooling to 0° the precipitated protein was removed by centrifugation. 1 ml of 50% diethylamine dissolved in acetone was then added per ml of the supernatant and the blue colour which developed was measured at 564 mμ. Duplicate determinations were carried out each time, the difference between readings being less than 10 per cent.

RESULTS

It appears (Table 1) that the quantity of Degranol decreased more rapidly in the resistant than in the sensitive tumour. In Degranol-sensitive tumours after 1–2 hr incubation there was 66 per cent of

TABLE 1.

Tumour tested*	Incubation (hr)	Concentration of Degranol μg/ml incubated			
		Ascitic fluid containing tumour cells	Ascitic fluid without tumour cells	Physiological saline	Physiological saline containing tumour cells
S	0	100	100	100	100
R	0	100	100		100
S	1	66	78	100	91
R	1	47	75		62
S	2	63	60	100	86
R	2	39	57		50
S	3	46	50	100	79
R	3	29	52		48
S	4	48	58	100	68
R	4	34	59		43
S	6	35	45	100	48
R	6	20	47		22
S	18	20	29	100	41
R	18	9	28		20
S	24	11	12	100	39
R	24	7	11		18

* *S* — Degranol-sensitive, *R* — Degranol-resistant.

the amount seen at 0 hr, while in the resistant tumour the amount at 1–2 hr was 47–39 per cent. This difference of 20 per cent was found at each time of testing. It was also of interest to determine whether this observed difference between the two tumours was due to the ascitic fluid or due to the tumour cells. In these experiments ascites was taken from the animals and the tumour cells immediately removed by centrifugation. To the ascitic fluid obtained in this way from both sensitive and resistant tumours, Degranol (100γ/ml) was added and its rate of disappearance followed. Alternatively Degranol was added to a suspension of washed tumour cells in saline and, its disappearance again followed. The rate of disappearance of Degranol in saline was also measured.

It was found that Degranol incubated in saline showed no decomposition after 24 hr. In ascitic fluid free from tumour cells, no difference was observed in the rate of disappearance of Degranol. However, in the preparation containing tumour cells free from ascitic fluid and suspended in saline, there was a greater decrease in Degranol concentration with resistant tumour cells than with sensitive cells.

DISCUSSION

In the tumour showing acquired resistance to Degranol, this drug disappears from the ascitic fluid quicker than in the sensitive tumour, either because the drug is more quickly decomposed, or because it binds more to the resistant tumour cells. Since in cell-free ascitic fluid, the rate of disappearance of Degranol is the same for both tumours, the first possibility is excluded. The observed difference is probably due to the fact that more Degranol is bound to resistant than to sensitive cells. This phenomenon has been observed with tumours resistant to antimetabolites and to alkylating agents. For example, 100 times more 8-azoquanine is bound to the RNA of the resistant tumour than to the sensitive tumour.[5] In rats bearing a sensitive and a resistant sarcoma inoculated bilaterally, ten times more Sarcolysine was bound to the DNA of the resistant tumour than to the sensitive.[6] It is assumed that increased binding is accompanied by an increased inactivation of the agent and this may serve as an explanation of the mechanism of resistance.

SUMMARY

The rate of disappearance of Degranol during incubation with sensitive and resistant NK/lymphoma ascites tumours has been measured. In sensitive tumours the concentration of Degranol decreased by 50 per cent in 3–4 hr and at 24 hr only 10 per cent remained. In the resistant tumour the quantity of active alkylating agent decreased by 50 per cent in 1 hr and only 20 per cent was present at 6 hr. At any time there was 20 per cent less Degranol in the ascitic fluid of the resistant tumour than in the sensitive. It is suggested that the cells of the resistant tumour bind more Degranol than the cells of the sensitive tumours.

Oncopathological Institute,
Budapest, Hungary.

ÉVA GÁTI
ZOLTÁN KÖRÖS

REFERENCES

1. Z. KÖRÖS, F. HARTAI, U. MÁTÉ-WOJCINSKA and C. SELLEI, Beitrage zum Wirkungsmechanismus des Degranols. *Z. Krebsforsch.* **66**, 374 (1964).
2. O. KLATT, A. C. GRIFFIN and J. S. STEHLIN. Method for determinations of phenyl-alanine mustard and related alkylating agents in blood. *Proc. Soc. exp. biol. Med.* **104**, 629 (1960).
3. P. OBRECHT, J. W. WOENCHHAUSE and K. H. STRICKSTROCK. Sum Nachweis von N-Methyl-bis-chloäthylamin/Dichloren/im Organismus der Ratte durch 4-p-Nitrobenzylpiridin/NBP/. *Z. Krebsforsch.* **66**, 161 (1964).
4. É. GÁTI. Morphologische und enzymchemische Veränderungen an einem Mause-Asciteslymphom mit indizierter Reistenz. *Z. Krebsforsch.* **65**, 506 (1963).
5. H. SKIPPER. On the mechanism of action of certain temporary Agents. *Canc. Res.* **13**, 545 (1963).
6. M. A. NOVIKOVA. *Fate of Sarcolysine C_{14} in Tumour-Bearing Animals.* VIII. International Cancer Congress 336 Medgiz.