MITOSIS OF CHICK FIBROBLASTS IN THE PRESENCE OF UNSATURATED IMIDES AND SULPHYDRYL COMPOUNDS

by

E. FRIEDMANN, D. H. MARRIAN

AND

I. SIMON-REUSS

Department of Radiotherapeutics, Cambridge University, Cambridge (England)

INTRODUCTION

We have reported previously that the unsaturated imides, maleimide, citraconimide and N-ethylmaleimide, have antimitotic properties, when tested in tissue cultures of chick fibroblasts, and that they share with other mitotic inhibitors, investigated by us —*i.e.*, maleic acid and the 1:4-naphthoquinones—the property of reacting with –SH compounds (FRIEDMANN, MARRIAN AND SIMON-REUSS[1,2,3]).

The investigation of the –SH addition products of the quinones and of maleic acid gave, as pointed out[2], apparently contradictory results, as in some of the adducts the antimitotic activity of the parent substances was abolished while others exerted antimitotic activity. Thus the thioethers, formed by the interaction of the thiols with quinones and maleic acid, were in a number of cases new mitotic inhibitors (FRIEDMANN[4]). The investigation of the quinone and the maleic acid group, therefore, afforded no explanation for the parallelism between mitotic inhibition and the –SH uptake, encountered in our investigations.

We hoped to advance this problem by studying the –SH addition products of the unsaturated imides, mentioned above, in their action on the mitosis of chick fibroblasts. The results obtained in this respect were no different from those obtained with the –SH adducts of the quinones and of maleic acid, but the analysis of these experiments brought a factor to light which so far had not been observed.

METHODS

The experiments were carried out in tissue cultures of chick fibroblasts, using the *hanging drop method*, as described previously[1].

Adducts of thiclacetic acid to N-ethylmaleimide (MARRIAN[5]), and the reaction product of thiourea with N-ethylmaleimide (MARRIAN[6]) have been isolated. These crystalline compounds were used in the tissue cultures. Other –SH addition products were obtained by mixing equimolar solutions of the imides with the corresponding thiol, neutralised to phenol red and adjusted to pH 7.4 with phosphate buffer. At the beginning of our experiments the concentrations of these mixtures before dilution to the concentrations used in the tissue cultures were $M/1000$. In the course of our investi-

References p. 64.

gations we obtained different results with glutathione when we used dilutions starting at $M/1000$ from dilutions starting at $M/50$. Using glutathione as thiol compound, therefore, two sets of experiments were carried out. In one set the components were mixed to give a molar concentration of 1/50 and in the other set to give 1/1000 molar concentration of the reactants. The solutions so obtained were diluted with Tyrode to give the concentrations to be tested in the tissue culture experiments.

RESULTS

The results obtained fall into two groups. In group (1) maleimide, N-ethylmaleimide and citraconimide were tested in the presence of thiolacetic acid. The compounds obtained from the interaction of thiourea and N-ethylmaleimide can be considered with this group. Group (2) contains the results, obtained with the adducts of glutathione to maleimide, N-ethylmaleimide and citraconimide.

In group (1) no mitotic inhibition was found with the adducts of thiolacetic acid to maleimide, nor with the crystalline addition product of thiolacetic acid to N-ethylmaleimide, or the crystalline substance resulting from the interaction of thiourea with N-ethylmaleimide. The antimitotic activity of maleimide and of N-ethylmaleimide is thus abolished by the addition of thiolacetic acid or thiourea to these unsaturated imides. Different results were obtained with the adduct of thiolacetic acid to citraconimide. Here mitotic inhibition of 50% was found at $8 \cdot 10^{-6} M$ concentration. As the adduct of thiolacetic acid to citraconimide has not been isolated, the question remains open as to whether the antimitotic activity is due to the reaction product or to the incomplete interaction of citraconimide with thiolacetic acid described previously[3].

In group (2) the action of glutathione on the mitotic inhibition produced by maleimide, N-ethylmaleimide and citraconimide was studied. The mixtures prepared at 1/50 and those prepared at 1/1000 molar concentration gave different results. The solutions obtained by diluting the mixture of 1/50 molar concentration of the reactants gave a mitotic inhibition which was considerably smaller than the mitotic inhibition of the original imides, as seen from Table I.

TABLE I

MITOTIC INHIBITION OF SOME IMIDES AND OF THEIR MIXTURES WITH GLUTATHIONE REACTIONS CARRIED OUT AT $M/50$

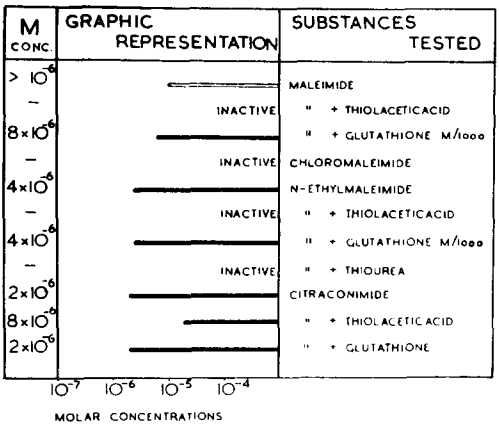
Substances tested	*Molar Concentration*	*Mitotic Inhibition*
Maleimide	$5 \cdot 10^{-6}$	21.6%
Maleimide + glutathione, $M/50$	$5 \cdot 10^{-6}$	0
Citraconimide	$3 \cdot 10^{-6}$	61.8%
Citraconimide + glutathione, $M/50$	$3 \cdot 10^{-6}$	37.0%
N-Ethylmaleimide	$2 \cdot 10^{-6}$	50.0%
N-Ethylmaleimide + glutathione, $M/50$	$2 \cdot 10^{-6}$	18.6%

On the other hand, the solutions prepared from a mixture of 1/1000 molar concentration were as active as the original inmides. Table II demonstrates these results.

References p. 64.

TABLE II

50% MITOTIC INHIBITION IN THE MALEIMIDE SERIES



Tissue cultures: chicken fibroblasts.
——— values numerically ascertained;
□□□□ values numerically not ascertained.
"Inactive" stands for "no activity at 10^{-5} *M* concentration and greater dilutions".

DISCUSSION

As the reaction between glutathione and maleimide or N-ethylmaleimide goes to completion at *M*/50 one is justified in interpreting the results obtained with dilutions starting from 1/50 molar concentration of the reactants as due to the adducts. The same applies for the dilutions starting from 1/1000 molar concentration, as the –SH uptake at 1/1000 final molar concentration of the reactants was found in four determinations to be in two minutes 98.2% (± 0.01%)[8].

Investigations are in progress to find an explanation for the effect of dilution on the antimitotic activity of the adducts of glutathione on unsaturated imides and to prepare these compounds. In view of the important application which the reaction of unsaturated imides with –SH compounds has found in biochemical experiments (HAYNES, HIND AND ISHERWOOD[7]), we believe it will serve a useful purpose to communicate the well ascertained biological observations described in this paper, and to leave the chemical explanation of these results for future work.

ACKNOWLEDGEMENT

One of us (E.F.) is indebted to Messrs. May and Baker, Dagenham, Essex, for financial support.

SUMMARY

1. In tissue cultures of chick fibroblasts thiolacetic acid abolishes the antimitotic activity of maleimide and N-ethylmaleimide, but citraconimide remains active as a mitotic inhibitor in spite of the presence of thiolacetic acid. The compound obtained from the interaction of thiourea with N-ethylmaleimide is inactive.

References p. 64.

2. Glutathione gives different results when diluted after interaction with maleimide, N-ethylmaleimide and citraconomide at $M/50$ and at $M/1000$. The solutions obtained from $M/50$ have decreased antimitotic activity, compared with the antimitotic activity of the original imides, whilst the dilutions prepared from $M/1000$ are as active as the original imides.

RÉSUMÉ

1. Dans les cultures de tissus l'acide acétique sulfhydrylé dépouille la maléimide et la N-éthylmaléimide de leurs activités antimitotiques. La citraconimide reste active comme inhibiteur mitotique malgré la présence de l'acide acétique sulfhydrylé. Le produit de réaction entre la sulfo-urée et la N-éthylmaléimide est inactif.

2. Le glutathion donne des résultats différents, dépendants de la concentration à laquelle il a réagi avec les imides. Les solutions qui proviennent de la réaction, exécutée à la concentration moléculaire de 1/50, ont une activité diminuée, comparée à celle des imides originales, tandis que la concentration moléculaire de 1/1000 fournit des solutions qui sont aussi actives que les imides mères.

ZUSAMMENFASSUNG

1. Thiolessigsäure hebt in Gewebekulturen von Hühner-Fibroblasten die antimitotische Wirkung des Maleinimids und des N-Äthylmaleinimids auf. Citraconimid bleibt trotz der Anwesenheit von Thiolessigsäure activ als mitotischer Hemmungsstoff. Das Reaktionsprodukt von Thioharnstoff mit N-Äthylmaleinimid ist unwirksam.

2. Glutathion gibt verschiedene Resultate, wenn seine Lösungen nach Reaktion mit Maleinimid und N-Äthylmaleinimid bei $M/50$ hergestellt werden, und wenn sie der Reaktion bei $M/1000$ entnommen werden. Die Lösungen, die der Reaktion bei $M/50$ entstammen, haben im Vergleich mit der antimitotischen Wirkung der ursprünglichen Imide verminderte Aktivität, während die Reaktion bei $M/1000$ Lösungen liefert, die ebenso wirksam wie die der ursprünglichen Imide sind.

REFERENCES

[1] E. FRIEDMANN, D. H. MARRIAN, AND I. SIMON-REUSS, *Brit. J. Pharmacol.*, 3 (1948) 263.
[2] E. FRIEDMANN, D. H. MARRIAN, AND I. SIMON-REUSS, *Brit. J. Pharmacol.*, 3 (1948) 335.
[3] E. FRIEDMANN, D. H. MARRIAN, AND I. SIMON-REUSS, *Brit. J. Pharmacol.*, 4 (1949) 105.
[4] E. FRIEDMANN, *Bull. soc. chim. biol.*, 31 (1949) 506.
[5] D. H. MARRIAN, *J. Chem. Soc.*, (1949) 1515.
[6] D. H. MARRIAN, *J. Chem. Soc.*, (1949) 1797.
[7] C. S. HANES, F. J. R. HIND, AND F. A. ISHERWOOD, *Nature* (London), 166 (1950) 288.
[8] E. FRIEDMANN – Unpublished.

Received September 11th, 1951