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PROTECTION BY CYSTEINE AGAINST THE ACUTE TOXICITY OF A CHEMICAL RADIO-SENSITIZER ("SYNKAVIT")

BY

A. F. PHILLIPS* AND D. B. CATER†

From the Departments of Radiotherapeutics and Pathology, University of Cambridge

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The vitamin K substitute tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate ("Synkavit") has been shown to act as a radio-sensitizer in tissue culture (Mitchell and Simon-Reuss, 1947, 1952) and in the radiation treatment of certain tumours in rats and in human patients (Mitchell, 1953a, 1955). Mitchell (1953b) suggested that the radiosensitization might be due to an interaction with thiol groups.

Synkavit, injected intravenously, lowers oxidation-reduction potentials in rat muscle and tumours (Cater and Phillips, 1954) and in all other tissues that have been tried (Cater and Phillips; Silver, Cater, and Phillips; in preparation). Several drugs that protect against radiation, including cysteine, cause a fall in these potentials in tumour, muscle, and lactating mammary gland, but cysteine causes a rise in cerebral grey matter. These observations led us to inquire whether cysteine would potentiate or antagonize the acute toxic effects of synkavit.

METHODS

White Wistar rats weighing between 140 and 230 g. were used. Males and females were kept separately and were used in different experiments. In any one experiment, rats of one sex were weighed and then allocated to experimental groups so that the rats of each group had approximately the same distribution of weights. Care was taken to avoid any bias in this process, which was preferable to strict randomization because of the wide range of weights and the small numbers in the groups. After allocation to their groups, rats were kept singly in individual boxes, without food or water, until about 4 hours after the last injection; they were then returned to cages, in their groups, with free access to food and water.

In every experiment but one, cysteine was injected first. It was weighed as hydrochloride, dissolved in boiled distilled water, and (in most experiments) partly neutralized to *p*H 3–6 with boiled N-NaOH immediately before injection. Under these conditions there was no visible precipitation of cystine for many minutes. The total volume injected was 0.6–1.0 ml. Unprotected groups were injected with a similar volume of isotonic saline. Injections were made into the lateral tail vein after 1 min. immersion in warm water. After the required interval, tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate ("Synkavit" Roche, 0.3 M) was injected into the opposite tail vein. Dosage of each drug was proportional to body weight.

The time of survival after the synkavit injection was recorded. Nearly all the rats that were injected with synkavit alone either died within 45 minutes or survived for at least several hours. The first assessment of the effect of an injection sequence was made on the proportion of deaths within 45 minutes. Results assessed in this way are presented in Table I. An assessment was also made on the basis of the mean reciprocal survival time for each group of rats. This particular transformation was chosen to allow the inclusion of individuals that survived indefinitely (Gaddum, 1953), and it proved to be the most convenient assessment for much of the data (Fig. 1).

RESULTS

For the first series of experiments a dose of synkavit, 1.3 mM/kg., was chosen to give 100% mortality within 45 min. Marked protection was obtained when an equimolecular proportion of cysteine hydrochloride was injected 3 min. before or 3 min. after the synkavit. There was still protection when the same dose of cysteine was administered up to an hour before the synkavit. Three times the dose of cysteine, neutralized, gave protection at 1 hour and at 3 hours, and also apparently at 24 hours, although subsequent experiments with the same and different doses of synkavit failed to confirm any protection at 24 hours.

In the second series of experiments the same dose of synkavit was used, but the rats had a higher susceptibility to the drug. Very marked protection

* Saltwell Scholar of the Royal College of Physicians.
† British Empire Cancer Campaign Research Fellow.

was still found with the larger dose of cysteine given one hour before the synkavit.

The results for these two series are summarized in Table I.

In the third and longest series of experiments, the susceptibility to synkavit (alone) remained fairly constant. The LD50, for death within 45 minutes, was approximately 60 mg. (free ester)/200 g. rat (0.9 mM./kg.). Several doses of synkavit were used, with small groups of rats, while the protected groups had the same or larger doses of synkavit; the dose of cysteine (partly neutralized) was always 3.9 mM./kg., given one hour before the synkavit. Very marked increase in survival times was found, with no deaths within 45 minutes even at doses of synkavit over twice the LD50. The results of this series are summarized graphically in Fig. 1.

A similar experiment was carried out using glutathione at a dose of 1.6 mM./kg. The dotted lines in Fig. 1 refer to this experiment, and show an increase in survival times for the protected groups. A single group, matched with the same control

TABLE I

PROTECTION BY CYSTEINE AGAINST A LETHAL DOSE OF SYNKAVIT (1·3 mM./kg.)

Cysteine		No. of Rats	Deaths Within 45 min.	Alive and Well at 48 hr.	*P*
mM./kg.	Time				
First series					
0·0		27	27	0	—
1·3	3 min. before	8	1	6	0·000001
1·3	3 ,, after	5	0	4	0·000005
1·3	½ hr. before	6	2	1	0·0004
1·3	1 ,, ,,	5	2	3	0·0020
3·9	1 ,, ,,	5	0	0	0·0004
3·9	3 ,, ,,	5	2	0	0·0020
3·9	24 ,, ,, (i.p.)	8	5	2	0·0036
Second series					
0 0		8	8	0	—
1·3	1 hr. before	9	8	0	—
1·3	3 ,, ,,	5	5	0	—
3·9	1 ,, ,,	7	0	1	0·0002
3·9	3 ,, ,,	6	6	0	—
Control: No synkavit 4·7 mM./kg. cysteine, followed by saline		6	0	6	—

P, the probability of getting a particular result by chance, has been calculated by the exact method for 2 × 2 tables with fixed marginal totals (Fisher, 1946). Each protected group has been compared with the unprotected group of the same series, taking account of deaths within 45 minutes only.

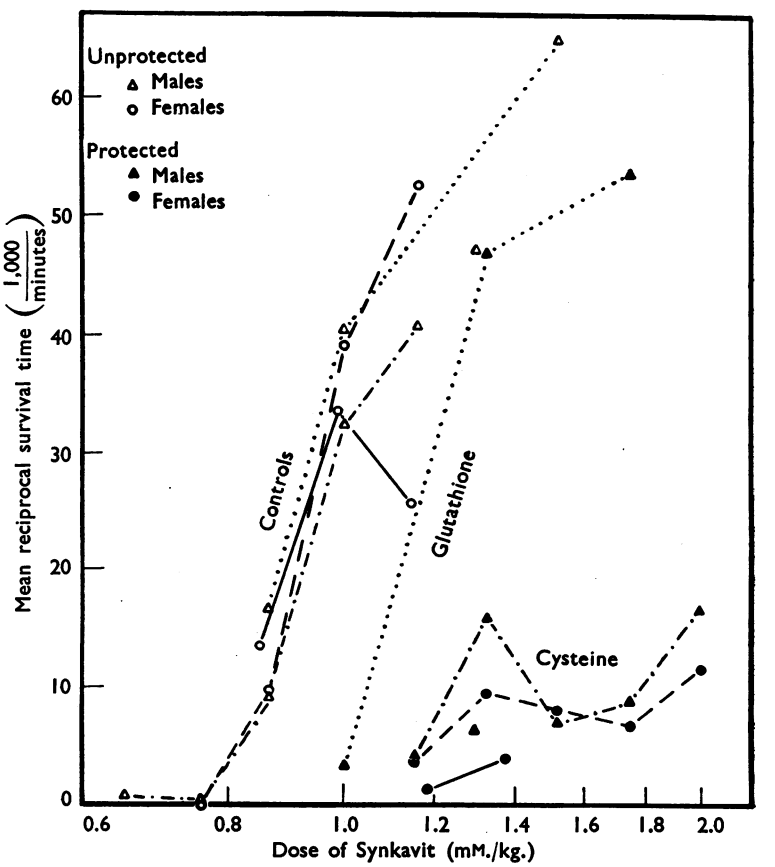

FIG. 1.—Effect upon reciprocal survival time of different doses of "Synkavit" administered alone, and one hour after protection with either cysteine (3·9 mM./kg.) or glutathione (1·6 mM./kg.). Each point represents the mean value for a group of rats (average 6 rats per group). Each set of open symbols joined by a line, together with the corresponding set of solid symbols, represents one experiment with groups matched for weight distribution, and used on the same day. (In one of the experiments there was only one protected and one unprotected group, which are represented by two isolated points.)

groups, received 3.3 mM./kg. of glutathione followed by synkavit, 1.3 mM./kg. The survival time was further increased, but was shorter than with cysteine at a dose of 3.9 mM./kg. Owing to the high cost of pure glutathione, the groups in this experiment were only of 3 rats each.

A control group of rats received neutralized cysteine alone, 4.7 mM./kg.; and another small group received the synkavit solvent alone (kindly supplied by Dr. A. L. Morrison of Roche Products, Ltd.), in amount corresponding to twice the largest dose of synkavit. Neither of these groups showed more than a transient malaise, and both appeared perfectly well before they were killed. Careful naked-eye post-mortem examination revealed no abnormality.

No differences attributable to sex were found either with synkavit alone or with cysteine plus synkavit. No trend of mortality or survival time with body weight was apparent for protected or unprotected rats, showing that the method of adjusting dose to body weight by simple proportion was satisfactory. Further analysis of the pooled "unprotected" results showed that this adjustment was more satisfactory than one depending on the 2/3 power of body weight or any lower power.

It was noted that unprotected animals frequently became very excitable shortly after the injection of a lethal dose of synkavit, and tended to leap against the sides of their cage. This excitable stage might proceed to convulsions before the animal died. In the protected animals the tendency to convulsions occurred later; the convulsions might start an hour after injection and last for 2 or 3 hours, before either death or recovery. Animals dying after a prolonged series of convulsions went into rigor mortis immediately.

We investigated the possibility that the convulsions might have been due to hypoglycaemia, and found that this was not so. Samples of blood (0.2 ml.) were taken from the tail veins of 5 rats, and kept for blood sugar estimation. Synkavit, 0.9 mM./kg., was then injected. After twenty minutes, 4 of the rats showed convulsions either spontaneously or in response to a sharp sound, and a second blood sample was taken from each by cardiac puncture. The blood sugar levels before injection ranged from 82–102 mg./100 ml.; and 20 minutes after injection they ranged from 131 to 199 mg./100 ml. The fifth rat, which had no convulsion, showed a rise of blood sugar from 92 to 284 mg./100 ml. in the 36 minutes after injection. Control estimations on normal rat blood before and after the addition of known amounts of synkavit, and of unphosphorylated 2-methyl-1: 4-naphthohydroquinone, showed that both substances, particularly the latter, could raise the reading obtained. The effect was not sufficient, however, to account for the whole of the observed rise after injection. There was, therefore, no fall of blood sugar to account for the convulsions.

Animals that died some hours after injection of synkavit, whether "protected" or not, frequently passed blood-stained urine, and post-mortem examination showed intense congestion of the kidneys. Sections of the kidneys showed great engorgement of the vessels and glomerular capillaries, and the presence of blood and protein exudates in the lumen of the tubules.

Discussion

Our results appear to be consistent with Mitchell's suggestion (1953b) that synkavit reacts with thiol groups in enzyme molecules. Friedmann (1954) showed that both cysteine and glutathione form addition products with synkavit, but we have no information on the toxicity of such compounds. Protection at as long an interval as 3 hours, with the relative molecular proportions used, would be difficult to explain on the basis of chemical neutralization of the synkavit in the blood.

The tendency of synkavit to cause convulsions suggests that the brain may be the organ most vulnerable to its toxic action. In this connexion it may be significant that we observed in brain a rise of oxidation-reduction potential after cysteine, and a fall after synkavit; whereas in other tissues both these drugs cause a fall.

In our unprotected groups, the LD50 for intravenous injection of synkavit (counting only deaths within 45 minutes) was 300 mg./kg. (expressed in accordance with the manufacturer's practice as free ester, mol. wt. 334). This may be compared with the LD50 found by Smith, Ivy, and Foster (1943) for rats, by subcutaneous injection, of 385 mg./kg. None of these rats died in less than 12 hours, and the average time of death was 30 hours. Foster (1940) also found LD50's of 280 mg./kg. for mice both by intravenous and by subcutaneous injection, and 425 mg./kg. for chicks by subcutaneous injection. (These values have been recalculated by us in terms of the free ester; in the works cited they were given in terms of the hydrated tetrasodium salt.) Smith *et al.* observed no excitability or convulsions in rats, but Foster records that a rabbit, injected intravenously with 200 mg./kg. of the tetrasodium salt (125 mg./kg. free ester), became excited, had convulsions at 30 minutes, and died 35 minutes after injection.

It is interesting that cysteine can protect against the toxic effects of nitrogen mustard (Nadkari,

Goldenthal, and Smith, 1954; Therkelsen, 1956), which has a radiomimetic action. We do not, however, regard synkavit as a radiomimetic drug, and we are not clear what relationship, if any, there is between these results and our own.

Summary

1. Tetrasodium 2-methyl-1 : 4-naphthohydroquinone diphosphate ("Synkavit") injected intravenously into rats had an immediate toxic action which caused death within 45 minutes. The LD50 was approximately 0.9 mM./kg. Rats which survived beyond 45 minutes usually lived for many hours or indefinitely.

2. Cysteine, injected at the same time or up to three hours before, in doses of 1.3 and 3.9 mM./kg., protected a high proportion of rats against the immediate toxic effect of synkavit, and increased the average survival times after doses of synkavit ranging from 1.0 mM./kg. to 2.0 mM./kg.

3. Cysteine was not itself toxic in doses up to 4.7 mM./kg.

4. Glutathione exerted a similar protective effect. The increase in survival time was comparable to that obtained with cysteine at the same molar dose.

5. Synkavit in toxic doses caused hyperexcitability and convulsions, which were not due to hypoglycaemia. This suggests an action on the central nervous system; oxidation-reduction potential measurements give some support to this view.

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References

Cater, D. B., and Phillips, A. F. (1954). *Nature, Lond.*, **174**, 121.

Fisher, R. A. (1946). *Statistical Methods for Research Workers*, 10th ed., p. 96. Edinburgh: Oliver and Boyd.

Foster, R. H. K. (1940). *Proc. Soc. exp. Biol., N.Y.*, **45**, 412.

Friedmann, E. (1954). *Brit. J. Radiol. (N.S.)*, **27**, 137.

Gaddum, J. H. (1953). *Pharmacol. Rev.*, **5**, 87.

Mitchell, J. S. (1953a). *Brit. J. Cancer*, **7**, 313.

—— (1953b). *Rep. Brit. Emp. Cancer Campaign*, **31**, 247.

—— (1955). *International Symposium of Radiobiology, Liége*, p. 170. London: Butterworth.

—— and Simon-Reuss, I. (1947). *Nature, Lond.*, **160**, 98.

—— —— (1952). *Brit. J. Cancer*, **6**, 305 and 317.

Nadkari, M. V., Goldenthal, E. I., and Smith, P. K. (1954). *Proc. Amer. Ass. Cancer Res.*, **1**, 33.

Smith, J. J., Ivy, A. C., and Foster, R. H. K. (1943). *J. Lab. clin. Med.*, **28**, 1667.

Therkelsen, A. J. (1956). *Progress in Radiology*, p. 260. Edinburgh: Oliver and Boyd.