THE EFFECT OF A SERIES OF ANTI-CANCER DRUGS ON PLASMA CHOLINESTERASE ACTIVITY*

ELEMER K. ZSIGMOND, M.D., AND GARY ROBINS, B.S.

ONE OF THE MOST frequently used muscle relaxants in anaesthetic practice is succinylcholine dichloride (suxamethonium, Sucostrin®, Anectine®, Quelicin®). The studies of Foldes *et al.*,[1] Evans *et al.*,[2] and Kalow *et al.*[3] have shown that plasma cholinesterase is primarily responsible for the metabolism of succinylcholine after its intravenous infusion in man.[4] Subsequently it became known that reduced plasma cholinesterase activity caused by liver disease, malnutrition, or cachexia results in prolongation of the neuromuscular block caused by succinylcholine[5] and in prolonged apnoea. Foldes *et al.* found a direct relationship between the reduction of plasma cholinesterase activity and the duration of apnoea caused by succinylcholine in patients with liver disease.[6] Therefore, it is likely that pathological conditions or drugs leading to reduced pseudocholinesterase activity may be responsible for a prolongation of the neuromuscular block after the intravenous injection of succinylcholine.

In this connection, Wang and Ross,[7] and Wolff[8] reported that cancer patients receiving anti-cancer agents, such as AB-132 or cyclophosphamide, were observed to develop prolonged apnoea after the intravenous infusion of succinylcholine. Both authors showed evidence that this prolongation of the effect of succinylcholine was caused by the inhibition of plasma cholinesterase by the anti-cancer drugs. In view of these findings, a systematic study was carried out to determine the *in vitro* inhibitory effect of a series of anti-cancer drugs on human pseudocholinesterase, as the first phase of investigations into this problem.

MATERIALS AND METHODS

The *in vitro* inhibitory effect of nine cytotoxic drugs on human plasma cholinesterase was determined by Kalow's ultraviolet spectrophotometric method[2] utilizing benzoylcholine chloride and procaine hydrochloride subtrates. For the experiments with benzoylcholine, a substrate concentration of 5.0×10^{-5} M and a final plasma dilution of 1:200 v/v were used. For the determination of the procaine hydrolysis rate, procaine hydrochloride in a concentration of 5.0×10^{-5} M and plasma in a final dilution of 1:10 v/v were employed.

Since the hydrolysis rate of succinylcholine can be determined less accurately than that of either benzoylcholine or procaine, the latter substrates were employed in this study. Earlier Foldes *et al.*[5] had reported that in patients with normal-

*From the University of Michigan Medical Center, Department of Anesthesiology, Ann Arbor, Michigan, USA and Anesthesia Research Laboratory of Allegheny General Hospital, Pittsburgh, Pennsylvania, USA.

This work was presented in part in a lecture at the Ninth Annual Meeting; American Society Clinical Pharmacology and Chemotherapy, May 1–3, 1969, Atlantic City, New Jersey, USA.

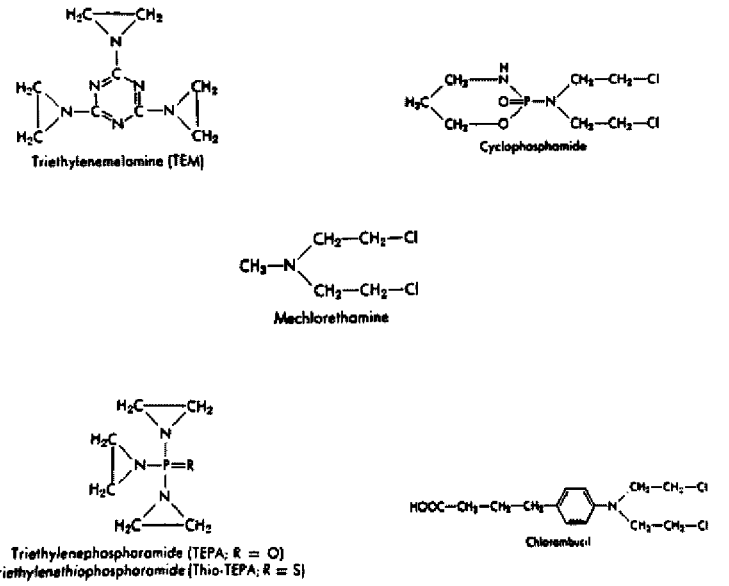

FIGURE 1. Structural formulae of the cytotoxic drugs studied. Alkylating agents.

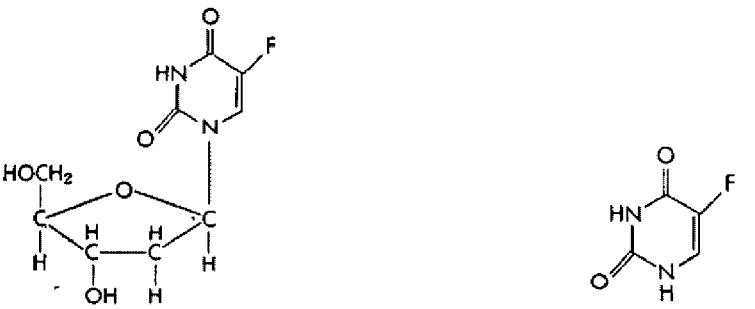

FIGURE 2. Structural formulae of the cytotoxic drugs studied. Antimetabolites.

homozygous plasma cholinesterase there are fixed ratios between the hydrolyses of benzoylcholine and succinylcholine and between procaine and succinylcholine. Therefore, any particular degree of inhibition of benzoylcholine or of procaine hydrolysis in the presence of plasma cholinesterase necessarily corresponds to a comparative inhibition of succinylcholine hydrolysis.

Among the nine anti-cancer drugs originally included were the alkylating agents Triethylene Melamine (TEM), Cyclophosphamide (Cytoxan), Mechlorethamine (Nitrogen Mustard), Triethylene thiophosphoramide (Thio-tepa), and Chlorambucil (Leukeran) (Figure 1). The anti-metabolites studied were: 5-Fluorouracil

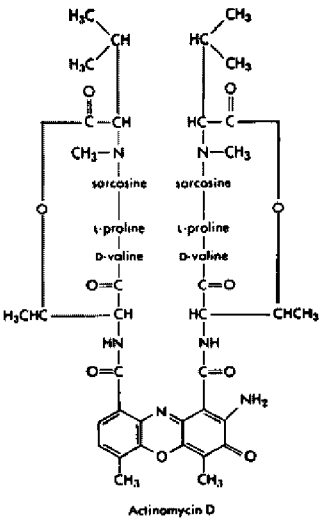

FIGURE 3. Structural formulae of the cytotoxic drugs studied. Antibiotic.

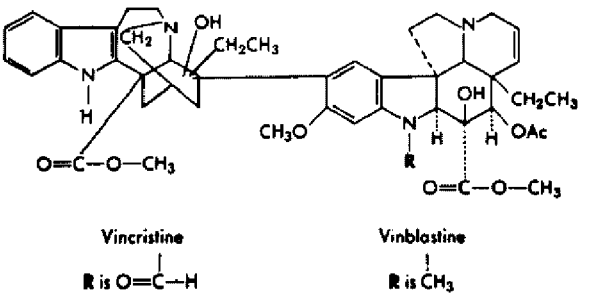

FIGURE 4. Structural formulae of the cytotoxic drugs studied. Vinca alkaloid.

and 5-fluoro-2′-desoxyuridine (Figure 2). In addition, Actinomycin-D (Figure 3) was considered and Vinblastine (Figure 4) was included. The limited solubility of Chlorambucil and Actinomycin-D interfered with the determination of their effect on plasma cholinesterase. Therefore, no data are available for these two anti-cancer agents.

The inhibiting effect of the anti-cancer drugs was studied with several concentrations sufficient to allow the plotting of the logarithm of the inhibitor concentrations against the percent inhibitions as shown in Figures 5, 6, 7, and 8.

RESULTS

Table I indicates that, of the anti-cancer drugs studied, only the alkylating agents and 5-fluoro-2′-desoxyuridine were soluble enough in the incubation me-

TABLE I

THE *In Vitro* INHIBITORY EFFECT OF CYTOTOXIC DRUGS ON HUMAN PLASMA CHOLINESTERASE

Name of drugs	I_{50} values of drugs	
	BeCh	Procaine
Alkylating agents:		
Triethylene Melamine	3.3×10^{-4} M	3.1×10^{-4} M
Cyclophosphamide	4.0×10^{-4} M	3.3×10^{-4} M
Mechlorethamine	6.3×10^{-4} M	5.6×10^{-4} M
Triethylene thiophosphoramide	7.9×10^{-3} M	7.9×10^{-3} M
Chlorambucil	NA*	NA*
Antimetabolites:		
5-Fluorouracil	>1.0×10^{-3} M†	>1.0×10^{-3} M†
5-Fluoro-2'-Deoxyuridine	No inhibition	No inhibition
Vinblastine	>1.0×10^{-4} M†	>1.0×10^{-4} M†
Actinomycin-D	NA*	NA*

*Solubility was inadequate for experiments.
†The use of higher concentrations was not possible because of its limited solubility in the incubation medium.

dium to permit determination of the I_{50} values, that is those concentrations which cause 50 per cent inhibition of enzyme activity. Although some inhibition of the plasma cholinesterase was observed at high concentrations of 5-fluorouracil, no inhibition of the enzyme occurred at all with 5-fluoro-2'-desoxyuridine even at a 1.0×10^{-2} M concentration.

Of the alkylating agents, the most potent anti-cholinesterase was triethylene melamine (TEM) as shown in Figure 5. Cyclophosphamide (Cytoxan) had

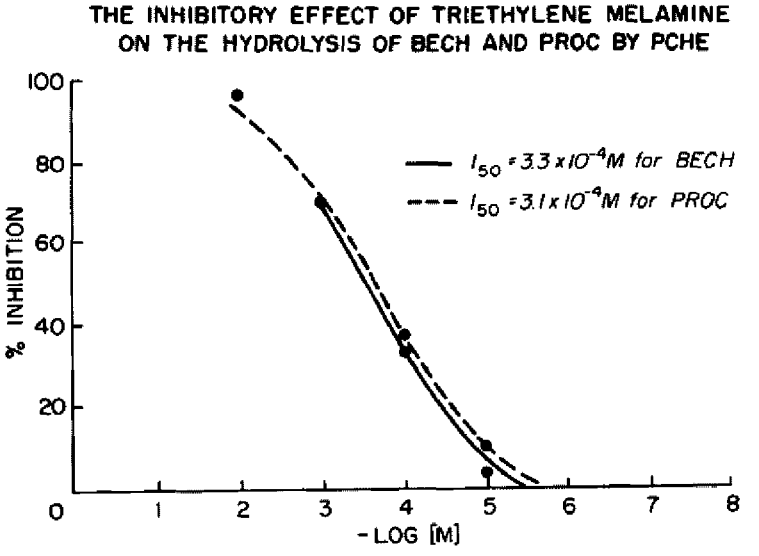

FIGURE 5

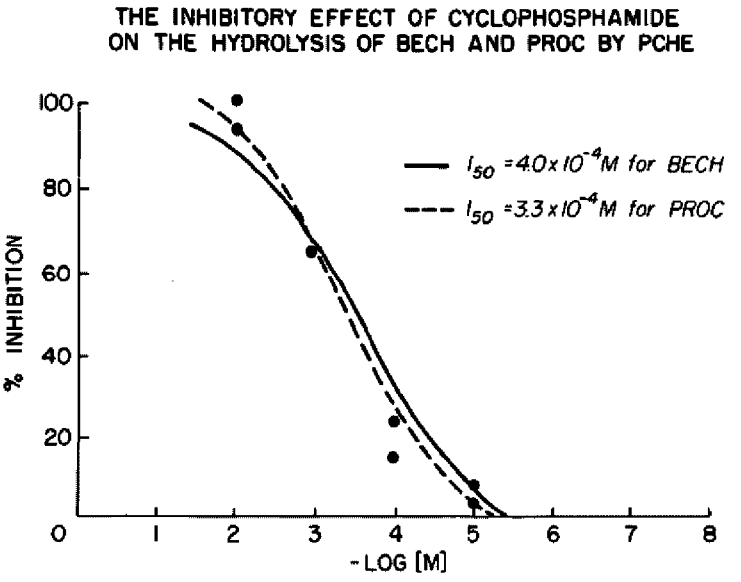

FIGURE 6

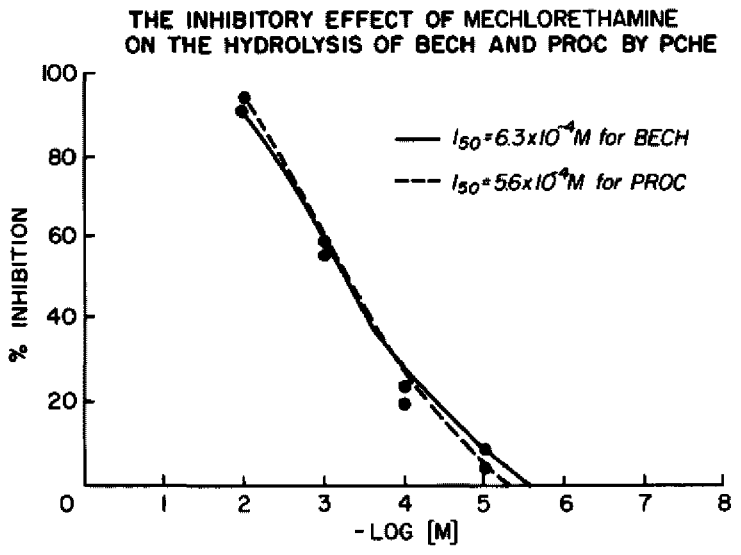

FIGURE 7

slightly lower inhibitory potency than triethylene melamine as shown in Figure 6. Mechlorethamine (Nitrogen Mustard) in turn has lower inhibitory potency than cyclophosphamide and this is demonstrated in Figure 7, while the anti-

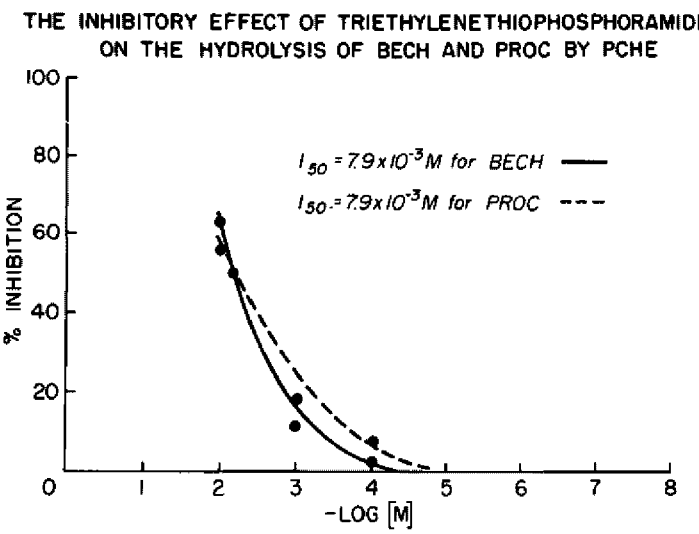

FIGURE 8

cholinesterase effect of triethylene thiophosphoramide (Thio-tepa) was negligible (Figure 8). The I_{50} values with benzoylcholine and procaine substrates were similar, indeed they were almost identical for all alkylating agents studied.

DISCUSSION

After large intravenous doses or with the prolonged administration of anti-cancer drugs possessing significant anticholinesterase effect such as triethylene melamine, cyclophosphamide and mechlorethamine, a marked reduction of plasma cholinesterase activity may occur. This may result in a reduced hydrolysis rate of succinylcholine. This inhibition of plasma cholinesterase may be of increased clinical significance in cancer patients who have been shown to exhibit frequently a reduction in plasma cholinesterase activity caused by the tumor itself.[7]

There is a linear correlation between the enzymatic hydrolysis rate of benzoylcholine as measured *in vitro* by Kalow's method and the duration of neuromuscular blockade caused by succinylcholine in man.[3,5] Thus, it is likely that a marked reduction in plasma cholinesterase activity caused by the alkylating agents may result in clinically significant prolongation of the neuromuscular blocking effect of succinylcholine. This in turn would result in prolonged apnoea. Because of the reduced break-down of succinylcholine, cardiac arrhythmias or even cardiac arrest are more likely to occur in terminal cancer patients whose enzyme activity may be reduced several-fold even before any treatment has been instituted. Therefore, in patients treated with triethylene melamine, cyclophosphamide, or mechlorethamine, the dose of succinylcholine and its rate of injection should be reduced in order to prevent respiratory depression and/or cardiac complications.

A controlled study of the effect of intravenously administered cyclophosphamide (250 mgm to 2.5 gm/70 Kg) on plasma cholinesterase activity is being con-

ducted currently on cancer patients. Preliminary results indicate a 35 per cent to 70 per cent reduction in the *in vivo* enzyme activity as determined *in vitro* immediately after completion of the injection. In some patients this inhibition lasts for several days. Since a reduction in plasma cholinesterase activity of greater than 70 per cent may result in a marked decrease in the *in vivo* hydrolysis of succinylcholine, individuals with less than one third of the normal activity are issued an identification tag which indicates their increased sensitivity to succinylcholine. In this way anaesthetic complications may be prevented. The final results of the studies on the *in vivo* plasma cholinesterase activity of cancer patients after the intravenous administration of cytotoxic alkylating agents will be reported in a forthcoming publication.

Summary

Prolonged apnoea caused by succinylcholine in combination with anti-cancer drugs in patients suffering from malignant tumors has been reported in past.

In order to determine the potential inhibitory effect of anti-cancer agents on plasma cholinesterase *in vivo* in cancer patients, a systematic study was carried out to determine their *in vitro* inhibitory effect. Utilizing Kalow's ultraviolet spectrophotometric method the hydrolysis of benzoylcholine and procaine by purified human cholinesterase and pooled human plasma was determined both in the presence and in the absence of anti-cancer agents. Of those studied, only the alkylating agents possess significant anticholinesterase effects. These are in decreasing order of effectiveness: triethylene-melamine (TEM), cyclophosphamide (Cytoxan), mechlorethamine (Nitrogen Mustard) and triethylene thiophosphoramide (Thio-tepa). The corresponding I_{50} values are 3.3×10^{-4} M, 4.0×10^{-4} M, 6.3×10^{-4} M, and 7.9×10^{-3} M concentrations with benzoylcholine as the substrate. In patients and especially in those treated with large intravenous doses of these anti-cancer drugs, the dose of succinylcholine should be reduced in proportion to the reduction of plasma cholinesterase activity to prevent prolonged apnoea and cardiac arrythmias which even may result in arrest. Therefore, patients who have more than 70 per cent reduction in plasma cholinesterase activity should be protected by wearing an Identification Tag.

Résumé

Dans le passé, il y a eu des rapports de publiés sur l'apnée prolongée causée par la succinylcholine chez des porteurs de tumeurs malignes sous traitement par des médications anticancéreuses.

De façon à déterminer l'effet inhibiteur possible des médications anticancéreuses sur la cholinestérase plasmatique in vivo chez les porteurs de cancer, nous avons fait une étude pour déterminer leur effet inhibiteur in vitro. Nous avons utilisé la méthode de Kalow : spectrophotométrie à l'ultraviolet et nous avons déterminé l'hydrolyse de la benzoylcholine et de la procaïne par de la cholinestérase humaine purifiée et par du plasma humain de plusieurs donneurs et, cela, en présence et en l'absence de médications anti-cancéreuses. Parmi les agents utilisés, seulement ceux qui ont tendance à produire des Alkyls ont eu des effets anticholinestérasiques importants. Voici, par ordre décroissant d'effet : le triethy-

lème-mélamine (TEM), le cyclophosphamide (cytoxan), le méchloréthamine (moutarde nitrogène) et le triéthylène thiophosphore amide (thio-tepa). Les valeurs correspondantes I_{50} sont 3.3×10^{-4} M, 4.0×10^{-4} M, 6.3×10^{-4} M et 7.9×10^{-3} M avec la benzoylcholine comme substrat. Chez les malades traités par des médications anti-cancéreuses et particulièrement chez les malades qui reçoivent de fortes doses de ces médicaments par voie endoveineuse, la dose de succinylcholine doit être réduite de façon proportionnelle à la réduction de la cholinestérase de manière à prévenir l'apnée prolongée, les arythmies cardiaques et, même, l'arrêt cardiaque. En conséquence, les malades dont l'activité cholinestérasique plasmatique est réduite d'au-delà de 70 pour cent, devraient porter un bouton d'identification pour leur sécurité.

ACKNOWLEDGEMENTS

We are indebted to Dr Sidney P. Shanor, Professor of Pharmacology, Duquesne University, Pittsburgh, Pennsylvania 15213, for his guidance of Gary Robins as his major advisor during his investigations.

We are grateful to the following pharmaceutical companies for their generous supply of anti-cancer drugs used in these studies; Lederle Laboratories, American Cyanamid Co. Inc. (Thio-tepa and Triethylene Melamine); Hoffmann-LaRoche Inc. (5-FU, FUDR); Burroughs Wellcome & Co. (Leukeran); Merck Sharp & Dohme (Actinomycin-D and Nitrogen Mustard); Mead Johnson (Cytoxan) and Eli Lilly Co. (Vinblastine).

REFERENCES

1. FOLDES, F. F., RENDELL-BAKER, L., & BIRCH, J. H. Causes and Prevention of Prolonged Apnea with Succinylcholine. Anesth. & Analg., *35*: 609 (1956).
2. EVANS, F. T., GRAY, P. W. S., LEHMANN, H., & SILK, E. Sensitivity to Succinylcholine in Relation to Serum Cholinesterase. Lancet, *1*: 1229 (1952).
3. KALOW, W. The Relation of Plasma Cholinesterases to Response to Clinical Doses of Succinylcholine. Canad. Anaesth. Soc. J., *3*: 22 (1956).
4. KVISSELGAARD, N. & MOYA, F. Estimation of Succinylcholine Blood Levels. Acta Anesth. Scandinav., *5*: 1 (1961).
5. FOLDES, F. F., FOLDES, V. M., SMITH, J. C. & ZSIGMOND, E. K. The Relation Between Plasma Cholinesterases and Prolonged Apnea Caused by Succinylcholine. Anesthesiology. *24*: 208 (1963).
6. FOLDES, F. F., SWERDLOW, M., LIPSCHITZ, E., VAN HEES, C. R., & SHANOR, S. P. Comparison of the Respiratory Effects of Suxamethonium and Suxethonium in Man. Anesthesiology, *17*: 559 (1956).
7. WANG, R. I. H. & ROSS, C. A. Prolonged Apnea Following Succinylcholine in Cancer Patients Receiving AB–132. Anesthesiology, *24*: 363 (1963).
8. WOLFF, H. The Inhibition of Serum Cholinesterase by Cyclophosphamid. Klin. Wschr., *43*: 819 (1965).

Abbreviations used in figures and tables:

PCHE = Acylcholine-acyl-hydrolase, plasma-cholinesterase, pseudocholinesterase
BeCh = benzoylcholine chloride

Chemical formulae of alkylating agents:

Triethylene melamine = 2,4,6-triethylene-imino-4-triazine
Cyclophosphamide = (2[bis (β-chloroethyl)amino] (2H)1,3,2-oxazaphosphorinane- 2-oxide
Mechlorethamine = Methyl-bis (β-chloroethyl)-amine
Chlorambucil = (4-[p-bis(β-chloro-ethyl)-aminophenyl] - butyric acid