J. Pharm. Pharmac., 1970, **22**, 738–744 *Received January* 19, 1970

Effects of some phenothiazines, tricyclic antidepressants and antihistamines on red cell agglutination

B. TAIT

Department of Pathology, The Royal Women's Hospital, Carlton, Victoria 3053, *Australia*

The observation that promethazine hydrochloride inhibited red cell agglutination in the ABO blood group system led to the screening of a series of drugs structurally related to promethazine in an attempt to establish a structure activity relation. Standard group O serum agglutinated group A and group B cells. The serum in each test was pretreated with drug and the degree of agglutination on the slide was assessed by both direct and microscopic means. All phenothiazines tested possessed this property except thiopropazate and the cyclic compound MO967C. Several tricyclic antidepressants and antihistamines tested possessed inhibitory activity, the tricyclic compounds were generally as active as the phenothiazines but the antihistamines varied in activity. The structure associated with maximum activity appeared to be a mono- or di-methyl substituted ethylamine or propylamine chain. However, the presence of the phenothiazine nucleus seems to strengthen activity suggesting that the overall shape of the molecule is important. Within the phenothiazine group there appeared to be a relation between basicity and inhibitory activity. It is suggested that the positively charged drug molecules in solution are attracted to the negative acidic groups on the red cell membrane thus inhibiting the formation of antibody-antigen complexes. The cyclization of the phenothiazine and the tricyclic compounds' side-chain appears to abolish the inhibitory action. These findings may be of clinical importance with respect to ABO incompatibilities between mother and foetus and also in laboratory serum antibody studies.

Promethazine inhibits agglutination in the ABO blood group system (Tait, 1968). This finding led to the investigation of other drugs having chemical structures or pharmacological activities related to those of promethazine, to determine the extent to which these factors were related to inhibition of red cell agglutination.

EXPERIMENTAL

Commercial preparations of human group A and group B cells suspended in a medium including inosine and disodium EDTA were supplied by the Ortho Pharmaceutical Company. Aliquots of suspensions of group A or group B cells were mixed separately on a slide with equal volumes of incompatible group O serum containing concentrations of drugs ranging from 5×10^{-11} to 5×10^{-2}M. One drop each of the cell suspension and of the treated serum were delivered from standardized pipettes. The rate and degree of agglutination were assessed by direct inspection and by microscopic examination of the mixtures on the slide, and were compared with control samples in which the drug solution was replaced by an equal volume of

0·9% NaCl solution. The ratio between successive concentrations of each drug was ten. The threshold concentration at which inhibition of agglutination was observed was recorded. Aqueous stock solutions or ampouled preparations of drugs were diluted in 0·9% NaCl solution for use in these tests.

RESULTS

Determination of the threshold concentration for inhibition of agglutination of group A and group B cells by group O serum with promethazine and the other drugs is illustrated in Fig. 1.

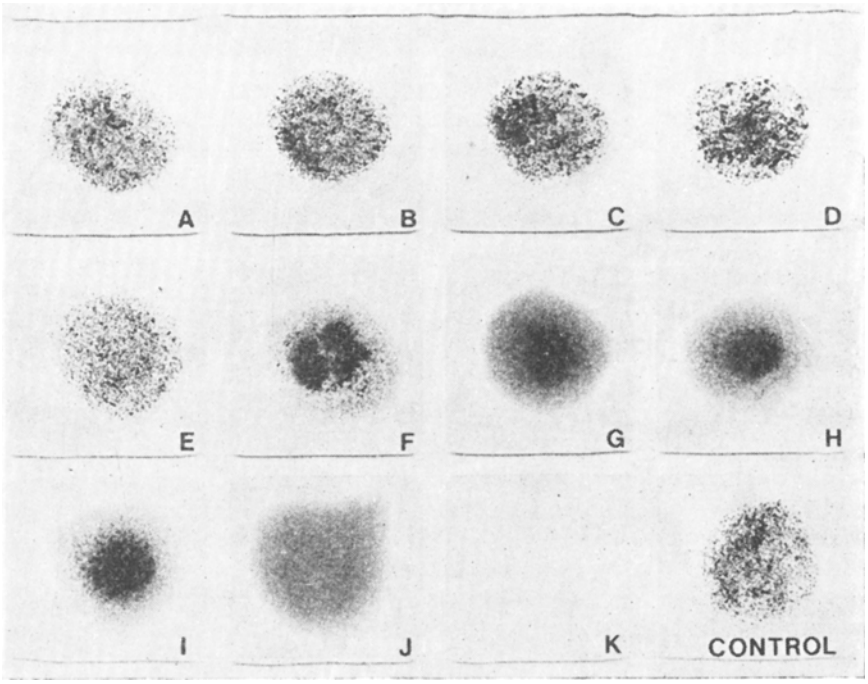
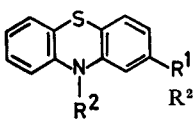


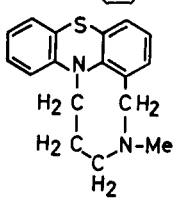
FIG. 1. The effect of a series of concentrations of promethazine hydrochloride on agglutination of a suspension of Group A red cells by Group O serum. The concentrations of promethazine ranged from 5×10^{-11}M in A to 5×10^{-2}M in J in ten-fold steps. In the control panel, the promethazine solution was replaced by saline. The threshold for inhibition of agglutination was 5×10^{-5}M with promethazine (G). The threshold concentration for haemolysis was 5×10^{-2}M (J).

Drugs used may be classified into the following four groups: (i) Phenothiazine derivatives with tranquillizing or sedative properties (Table 1). (ii) Tricyclic antidepressants which are considered to be structurally similar to the phenothiazines (Table 2). (iii) Drugs with antihistamine activity (Table 2). (iv) Drugs with structural similarities to promethazine, such as an ethylamine or propylamine chain and methyl or ethyl substitutions on the terminal nitrogen. The drugs in this group have diverse pharmacological activities.

Phenothiazine derivatives were found to be the most effective inhibitors of agglutination. Two (thioridiazine and thiethylperazine) were as potent as promethazine, but there was a wide range of threshold concentrations for inhibition of agglutination

Table 1. *Inhibition of agglutination by some phenothiazines*



Drug	R^1	R^2	Threshold concn (mM)
Thioridazine	–S—Me	–[CH_2]$_2$·(N–Me)	0·05
Thiethylperazine	–SO_2—CH_2—Me	–[CH_2]$_3$·N(N·Me)	0·05
Promethazine	–H	–CH_2·CH(Me)·NMe_2	0·05
Promazine	–H	–[CH_2]$_3$·NMe_2	0·5
Chlorpromazine	–Cl	–[CH_2]$_3$·NMe_2	0·5
Thioperazine	–SO_2—NMe_2	–[CH_2]$_3$·N(N·Me)	0·5
Fluphenazine	–CF_3	–[CH_2]$_3$·N(N·[CH_2]$_2$·OH)	0·5
Methdilazine	–H	–CH_2·(NMe)	0·5 (weak)
Carphenazine	–CO—CH_2—Me	–[CH_2]$_3$·N(N·[CH_2]$_2$·OH)	5 (weak)
Triflupromazine	–CF_3	–[CH_2]$_3$·NMe_2	5 (very weak)
Perphenazine	–Cl	–[CH_2]$_3$·N(N·[CH_2]$_2$·OH)	5 (very weak)
Thiopropazate	–Cl	–[CH_2]$_3$·N(N·[CH_2]$_2$·O·CO·Me)	N.I.
MO967C			N.I.

N.I. = No inhibition.

in this group of drugs (Table 1). Three phenothiazine drugs tested did not inhibit red cell agglutination: thiopropazate, MO 967C in which the side-chain is formed into a heterocyclic ring (Table 1), and methylene blue which has a phenothiazine nucleus.

The tricyclic antidepressants included three fairly potent inhibitors of agglutination (Table 2). Chlorprothixene was weak, and a cyclic derivative of imipramine was without activity.

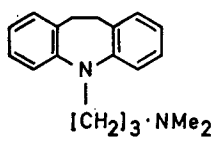
The activity of compounds with a cyclized side-chain, MO 967C and MO 795E differed from that of the respective parent open-chain compounds promazine and imipramine. The open-chain compounds inhibited agglutination with threshold

concentrations of 5×10^{-4} and 2×10^{-4}M respectively but the closed-chain compounds caused haemolysis, MO 967C at 5×10^{-4}M and MO 795E at $2{\cdot}5 \times 10^{-3}$M. This suggests that cyclization of the side-chain leads to loss of potency in inhibiting agglutination, but the extent of the loss could not be determined.

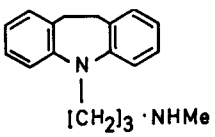
The antihistamines (Table 2), though inhibiting agglutination were generally less effective than the phenothiazine derivatives, many of which also possess antihistamine activity.

Atropine (25), butacaine (100), decamethonium (100), isoprenaline (100), methylene blue (50), neostigmine (100), oxyphencyclimine (25), penthienate (100), procaine (100), procaine amide (400), pronethalol (100), pyrididostigmine (100), fenoxazine (50), suxamethonium (100), trimethidinium (100), all failed to inhibit agglutination at the concentrations (mM) shown.

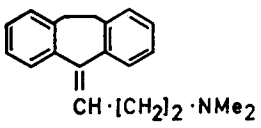
Table 2. *Inhibition of agglutination by some tricyclic antidepressants and antihistamines.* Threshold concentrations are given in parentheses



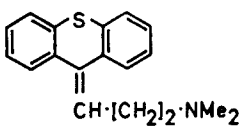
Imipramine
(0·2 mM)



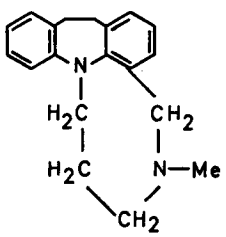
Desipramine
(0·5 mM)



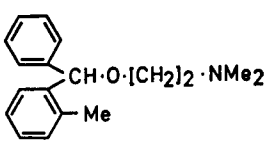
Amitriptyline
(1·5 mM)



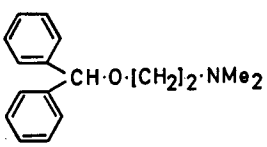
Chlorprothixine
(50 mM)



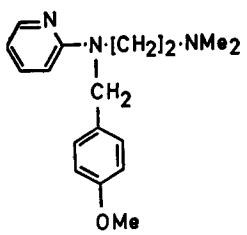
M0795E



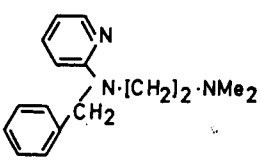
Orphenadrine
(0·3 mM)



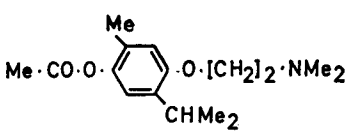
Diphenhydramine
(1·5 mM)



Mepyramine
(5 mM)



Pyribenzamine
(50 mM)



Thymoxamine
(50 mM)

DISCUSSION

The number of phenothiazine derivatives tested is too small to allow firm conclusions about correlation of structural characteristics with activity in inhibiting agglutination. However the nature of the chain attached to the nitrogen atom at the 10 position of the nucleus affects the properties of the drug. Thus thiopropazate had no activity and perphenazine was a weak inhibitor of agglutination whereas the analogous drug with a shorter chain, chlorpromazine, was a potent inhibitor. However trifluprazine was weaker than fluphenazine although it has a shorter chain. Foster & Fyfe (1966) reported that, as a result of stearic hindrance, long aliphatic side chains attached to the phenothiazine nucleus decrease the stability of electron donor acceptor complexes with 1,4-dinitrobenzene. The nature of the side-chain at the 2-position of the phenothiazine nucleus also influences the activity of the drugs in inhibiting agglutination. For example, thioperazine with a sulphonamide grouping on the phenothiazine nucleus was a weaker inhibitor of agglutination than the analogous compound thiethylperazine with a 2-ethylthio-grouping. There was also a tenfold difference in strength of inhibition between chlorpromazine (–Cl at the 2-position) and trifluprazine ($-CF_3$) the latter being much weaker. However, fluphenazine ($-CF_3$) was more potent than perphenazine.

Amongst the phenothiazines the weakest inhibitors are weak bases, but there was no consistent relation between pK_a and potency in inhibiting agglutination (Fig. 2). The major structural feature determining pK_a in phenothiazines is the nature of the 10-alkylamine side-chain (Green, 1967). A 2-halo-substituent may effect ionization

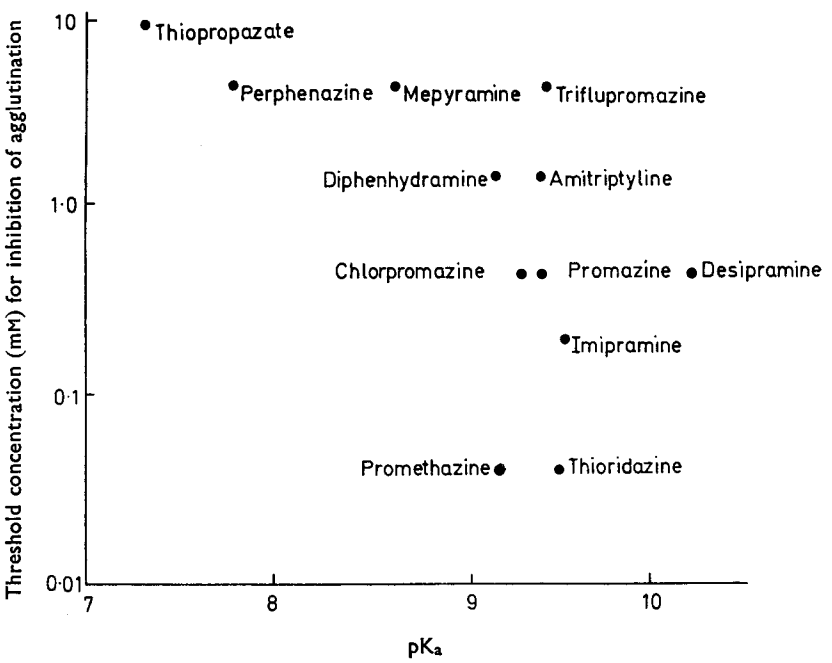


FIG. 2. Relation between potency in inhibiting agglutination and pK_a values for some phenothiazines, tricyclic antidepressants and antihistamines. Minimum concentrations required for inhibition of agglutination are expressed as $\log_{10}$ (100 × mM concentration). Not all the drugs used are included because of the absence of pK_a values of many in the literature.

but the conformation of the alkylamino-chain may remove it from this influence (Chatten & Harris, 1962). Strong bases with a quaternary nitrogen were inactive. The structure of the molecule rather than its degree of ionization appears to determine its ability to inhibit agglutination. However, it is likely that the cationic form of the drug molecule may be responsible for inhibition of agglutination, since acidic groups on the red cell membrane are incorporated in antigenic sites. That the drug attaches to the red cells is supported by the observation that promethazine-treated red cells washed several times in saline do not agglutinate as much as control cells which have not been exposed to the drug (unpublished observations). Irwin, Smith & Trams (1961) reported that gangliosides inhibit the action of chlorpromazine on muscle and suggested that this was due to combination of the drug and the acidic ganglioside rather than to an effect on drug receptors. They also suggested that neuraminic acid, which is present in gangliosides, was concerned in this interaction. Neuraminic acid is also present in the red cell membrane and may form part of the antigenic moiety (Cook, 1968).

The presence of *N*-methyl substituted alkylamine side-chain in phenothiazine, antidepressant and antihistamine drugs with activity in inhibiting agglutination suggests that this group may be important for the effect. The *NN*-dimethyl substituted ethylamine chain is common to most antihistamine drugs and is thought to be involved in binding at histamine receptor sites. Drugs with *NN*-diethyl substituted alkylamine chains such as procaine did not inhibit agglutination, possibly because the larger substituents on the nitrogen atom might prevent a close association of the cation with an anionic site on the red cell membrane.

Phenothiazine derivatives apparently show an inverse correlation of potency in inhibiting agglutination and tranquillizing potency. The most active drugs in inhibiting agglutination, such as thioridazine and promazine, are given in relatively large doses (75–200 mg in 24 h to ambulatory patients and 200–600 mg in 24 h to hospital patients). The weaker inhibitors of agglutination such as perphenazine are given in smaller doses (1–3 and 2–20 mg in 24 h). It is possible that the weaker inhibitors of agglutination are less firmly bound to red cell membranes and may be absorbed more readily into the central nervous system.

There are clinical implications to the observed inhibition of red cell agglutination by a number of drugs. Since the phenothiazine drugs are placenta permeable their administration during pregnancy in the case of foeto-maternal incompatibilities may help to lessen erythroblastosis at least when it is due to immune anti-A or anti-B antibodies. Bierme & Bierme (1967) observed that administration of promethazine in conjunction with intraperitoneal transfusions proved of benefit in cases of rhesus immunization. However promethazine *in vitro* has proved much less effective in inhibiting red cell agglutination due to Rh antibodies than that observed with the ABO system (unpublished observations).

Routine ABO grouping should come under closer scrutiny if the patients have been administered drugs which are potent in inhibiting agglutination. Although it is unlikely that a wrong ABO group determination will be obtained, the avidity of the reaction may be reduced. The use of some antihistamines in treating cases of transfusion reaction could be responsible for negative antibody tests on the slide, particularly if the antibody is weak, and could mask a genuine incompatibility reaction. The findings indicate the importance of taking blood samples for cross-matching and grouping, where practical, before drugs are given.

Acknowledgements

The author wishes to thank Professor M. J. Rand, Professor of Pharmacology, University of Melbourne for his supervision of this work. The author is also indebted to the following drug companies for the generous supply of drugs: Abbot Labs. Pty. Ltd., Amido Labs. Pty. Ltd., Bayer Pharmaceuticals Pty. Ltd., Geigy Aust. Pty. Ltd., ICI Ltd., May & Baker Pty. Ltd., Mead Johnson Pty. Ltd., Merck Sharp & Dohme Pty. Ltd., Parke Davis & Co., Pfizer Pty. Ltd., Riker Labs. (Aust.) Pty. Ltd., Roche Products Pty. Ltd., Sandoz Aust. Pty. Ltd., Schering Pty. Ltd., Searle, G. D. (Aust.) Pty. Ltd., Siquil Pty. Ltd., Smith, Kline & French Pty. Ltd., Squibb, E. R. & Sons Pty. Ltd., Winthrop Labs., Wyeth Pharmaceuticals Pty. Ltd.

REFERENCES

BIERME, A. & BIERME, R. (1967). *Lancet*, **1**, 574.
CHATTEN, L. G. & HARRIS, L. E. (1962). *J. analyt. Chem.*, **34**, No. 11, 1495–1501.
COOK, G. M. W. (1968). *Br. med. Bull.*, **24**, No. 2, 118–123.
FOSTER, R. & FYFE, C. A. (1966). *Biochem. Biophys. Acta*, **112**, 490–495.
GREEN, A. L. (1967). *J. Pharm. Pharmac.*, **19**, 10–16.
IRWIN, R. L., SMITH, H. J., TRAMS, E. G. (1961). *The Pharmacologist*, **3**, No. 2, 54.
TAIT, B. D. (1968). *J. Pharm. Pharmac.*, **20**, 479–481.