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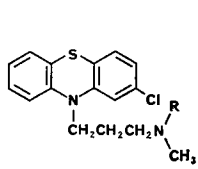
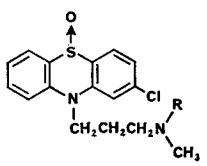
Chlorpromazine 'hydroxylamines' in red blood cells as major metabolites of chlorpromazine in man

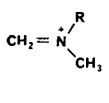
In man, 6–10 h after a single dose of 32 mg of chlorpromazine (Ia), about 10% of the dose is found in the red blood cells as a mixture of 'free' *N*-hydroxynorchlorpromazine (Ib) and its sulphoxide (IIb) in roughly equal amounts, while about 2% of norchlorpromazine (Ic) but only traces of the parent drug (Ia) and its *N*-oxide are present in these cells. At the same time, the plasma contains only about 0·5% of the dose as a mixture of the hydroxylamines (Ib) and (IIb) and less than a total of 0·3% of the parent drug and its *N*-demethylated derivatives.



I a R = CH_3
b = OH
c R = H



II a R = CH_3
b R = OH
c R = H



III a R = CH_3
b R = OH
c R = H

After 24 h, there is little change in the concentration of the hydroxylamines (Ib) and (IIb) in the cells but about 5% of norchlorpromazine (Ic) and negligible amounts of unchanged drug (Ia) are present; there is about 0·2% of (Ic) and negligible amounts of (Ia) in the plasma. After 13 days, the concentration in the cells of the hydroxylamines (Ib) and (IIb) is only slightly reduced and there is 0·5 to 1% of (Ib) in the plasma but neither parent drug (Ia) nor the demethylated compound (Ic) could be detected in cells or plasma.

Patients being treated with 300 to 600 mg of chlorpromazine (Ia) daily had concentrations of (Ib) and (IIb) in the cells that indicated a total amount of 20 to 40 mg in the cells and 5 to 10 mg of (Ic) but negligible amounts of the parent drug.

In addition to the above concentrations of "free" hydroxylamines (Ib) and (IIb), additional concentrations of conjugated forms, sometimes almost equal to those of the free forms, are present.

The free compounds were isolated by separately diluting red blood cells and plasma with water, complexing with methyl orange and selective extraction of these complexes by the control of pH and the use of different organic solvents. The organic extracts were concentrated under reduced pressure in the dark, in a nitrogen atmosphere, and then examined by t.l.c., g.l.c., polarography, mass spectrometry and for chemical reactions.

The metabolically produced hydroxylamines (Ib) and (IIb) gave results identical to those obtained using authentic synthetic material produced by careful oxidation of norchlorpromazine (Ic) by hydrogen peroxide or *m*-chlorperbenzoic acid.

Using t.l.c. silica gel, (Ib) and (IIb) gave R_F values 0·8 and 0·6 respectively [(Ia)

gave 0·8 and (Ic) 0·68] using benzene–methanol–diethylamine (75 : 15 : 10) (Beckett & Hewick, 1967); the R_F values of (Ib) and (IIb) were 0·76 and 0·36 respectively using chloroform–acetone (90 : 20) whereas (Ia) and (Ic) remained on the base line; the *N*-hydroxy didesmethylchlorpromazine had R_F 0·43. Ammoniacal silver nitrate gave a blue-grey colour with the spot of (Ib) and a slowly developing grey colour with the spot of (IIb), whereas the spots of (Ia) and (Ic) did not give any colour with this reagent.

Reaction of the hydroxylamine (Ib) with $TiCl_3$/HCl gave a slow reduction to norchlorpromazine (Ic) whereas (IIb) gave a quick reduction to (Ib) and slow reduction to (Ic).

(Ib) and (IIb) were reduced polarographically, reduction step −1·1 V in Walpole's acetate buffer pH 5.

Using the g.l.c. system: OV 17, 3% on Gas-Chrom Q (80–100); 1 m, 205°, nitrogen (carrier gas) flow rate 108 ml min^{-1}; the side chains of (Ib) and (IIb) were eliminated and the latter reduced to yield the 2-chlorophenothiazine nucleus (Rt 5·7), whereas chlorpromazine (Ia) and norchlorpromazine (Ic) could be chromatographed virtually unchanged by this system with Rt 11·8 and 14·6 respectively.

G.l.c.-mass spectrometry of (Ib) and (IIb) led to extensive fragmentation of the side chain; a characteristic *N*-hydroxylated fragment was present i.e. (IIIb) *m/e* 60 (Beckett, Coutts & Ogunbona, 1973). Under similar conditions, much less fragmentation of the side chain occurs with (Ia) and (Ic), the latter giving a characteristic fragment (IIIc) i.e. *m/e* 44, whereas (Ia) gave fragment (IIIa) with *m/e* 58 (Gilbert & Millard, 1969; Hammar, Holmstedt & Ryha, 1968).

The side chain attached to the phenothiazine nucleus is cleaved more rapidly under aqueous conditions in the form of the hydroxylamine (Ib) than as the bases (Ia) and (Ic); thus hydroxylamine formation may be the route to pigment formation from chlorpromazine in man since phenothiazines are more light sensitive and more rapidly oxidized to pigments than are their derived aminoalkylphenothiazine drugs.

The *N*-oxidation of many secondary and primary aliphatic amines has recently been reported (Beckett 1971; Beckett & Al-Sarraj 1972). Metabolic *N*-dealkylation of many tertiary aminoalkylphenothiazines is well known and therefore the formation of hydroxylamines from these metabolites of such phenothiazine drugs is to be expected; preliminary work using animal liver microsomes and various phenothiazines support this conclusion.

The significance of the extensive occurrence of 'hydroxylamines' of chlorpromazine and other phenothiazines in red blood cells is being investigated.

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