EFFECT OF CHLORPROMAZINE ON ENZYMIC OXIDATION OF LIPIDS

I. A. Eluashvili, T. P. Pashinova, E. D. Bogdanova, V. E. Kagan, and L. L. Prilipko

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Chlorpromazine was found to inhibit enzymic NADPH-dependent peroxidation of lipids in rat liver microsomes. The inhibitory effect was shown to be due to: 1) the antioxidant action of hydroxy derivatives of chlorpromazine produced in the course of its metabolism by NADPH-dependent microsomal oxygenases; 2) competition for reduced components of electron-transport chains between two NADPH-dependent processes — chlorpromazine metabolism and peroxidation of lipids.

KEY WORDS: *chlorpromazine; metabolism by multipurpose oxygenases; enzymic NADPH-dependent peroxidation of lipids.*

Preparations of the phenothiazine series, including chlorpromazine, are characterized by high effectiveness and, at the same time, a broad spectrum of pharmacological action. This action of chlorpromazine and products formed in the liver as a result of its enzymic metabolism via the monooxygenase pathway, may be based on two mechanisms: 1) interaction with several or many different targets in the cell; 2) activity aimed at one universal system.

One such universal system (the object for the action of chlorpromazine) may be the system of induction of peroxidation of lipids (POL), especially the enzymic NADPH-dependent system of POL localized in the membranes of the endoplasmic reticulum (EPR). This hypothesis seems even more natural because the universal role of POL processes in injury to biological membranes has now been conclusively proved [3].

Considering the evidence of the membranotropic action of chlorpromazine [4], in the investigation described below the effect of chlorpromazine on enzymic oxidation of lipids in rat liver microsomes was studied.

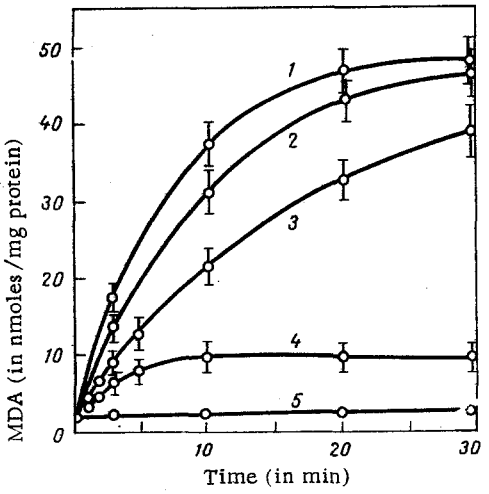

Fig. 1. Kinetics of accumulation of products active against thiobarbituric acid during enzymic and nonenzymic POL in presence of chlorpromazine and ionol. 1) Nonenzymic POL; 2) nonenzymic POL + chlorpromazine (10^{-4} M); 3) enzymic POL; 4) enzymic POL + chlorpromazine (10^{-4} M); 5) enzymic POL + ionol ($5 \cdot 10^{-5}$ M). MDA) Malonic dialdehyde.

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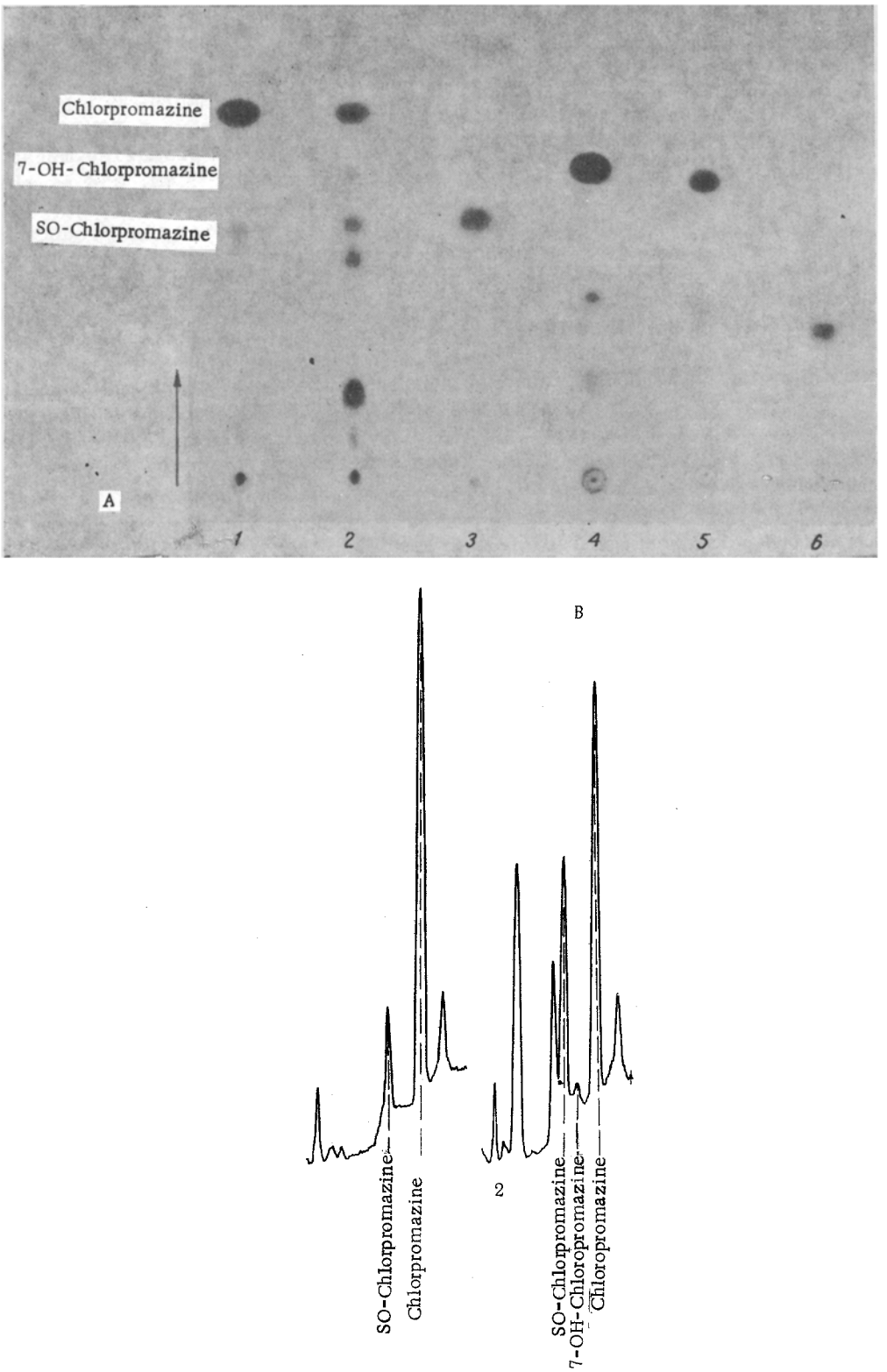

Fig. 2. NADPH-dependent chlorpromazine metabolism in rat liver microsomes. Typical chromatogram (A) and densitogram (B). 1) Control (0 min of incubation); 2) chlorpromazine metabolites formed after incubation for 20 min; 3, 4, 5, 6) standards: SO-chlorpromazine, 7-OH-chlorpromazine, 8-OH-chlorpromazine, and SO-Nor_2-chlorpromazine, respectively.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male albino rats weighing 150-200 g. The microsomal fraction was isolated from rat liver by Archakov's method [1]. The standard incubation medium contained 1 g microsomal protein in 1 ml, 0.5 mM NADPH, 0.1 M NaCl, 0.05 M Tris-HCl buffer, pH 7.0 (37°C). During analysis of ascorbate-dependent POL the NADPH was replaced by ascorbate (0.5 mM). In the experiments to study chlorpromazine metabolism $MgCl_2$ was added to the incubation medium up to a concentration of 3 mM, and in the experiments to study POL, $FeSO_4$ was added up to 10 μM. The reaction was started by the addition of NADPH and stopped with cold solutions of 30% TCA (for analysis of the products of POL) or methylene chloride (analysis of chlorpromazine metabolites). POL products were analyzed quantitatively by the method of Kohn and Liversedge [7]. To determine chlorpromazine and its metabolites, thin-layer chromatography was carried out on Silica Gel 60 with a layer 0.25 mm thick (Merck, West Germany). Chlorpromazine and its metabolites were extracted from the microsomal membranes twice with a mixture of methylene chloride and water (2:1) by the method of March et al. [8], and by chromatography twice in a mixture of isopropanol: ethanol:ammonia (22:2:1). For quantitative analysis of the chromatograms densitometry was carried out (with the Opton densitometer) in the ultraviolet region (255 nm). The chromatograms were developed with 50% H_2SO_4 + 5% $FeCl_3$ for visual control. The NADPH consumption was measured fluorometrically (Hitachi, MPF-2A) at wavelengths of 375 nm for excitation and 460 nm for emission. Chlorpromazine metabolites from Regis (USA) and Soviet chlorpromazine (aminazin) hydrochloride, the purity of which was verified chromatographically before each experiment, were used.

EXPERIMENTAL RESULTS

As Fig. 1 shows, chlorpromazine has an inhibitory action on enzymic POL which differs strongly from the inhibitory action of ionol (4-methyl-2,6-di-tert-butylphenol). Whereas ionol, in a concentration of 10^{-4} M, completely inhibits the formation of POL products from the very beginning of incubation, in the presence of chlorpromazine in the same concentration, inhibition of POL increases during the first 10 min of incubation and becomes complete after a further 10 min. The inhibitory action of chlorpromazine may be due to: 1) its antioxidant action; 2) monopolization by chlorpromazine of electron transport chains (ETC) and, consequently, competition for sources of reducing equivalents; 3) the formation of derivatives through NADPH-dependent metabolism of chlorpromazine which behave as antioxidants. The first hypothesis can be rejected, because in POL induced by an Fe^{2+}—ascorbate system (i.e., nonenzymic POL) chlorpromazine showed virtually no antioxidant activity (Fig. 1).

Considering that the inhibitory action of chlorpromazine on enzymic POL increases during the first 10 min of incubation, and that the chlorpromazine content decreases under these circumstances as a result of its NADPH-dependent metabolism (Fig. 2), it can be concluded that the mechanism of the inhibitory action of chlorpromazine is not simply one of competition for reduced components of the ETC. At the same time, it will be noted that after incubation for 10 min, i.e., toward the time when the inhibitory effect of chlorpromazine becomes maximal, the NADPH concentration in the system was reduced from 0.5 to 0.4 mM. Considering that the value of the Michaelis constant for enzymic POL in rat liver microsomes is 4 μM [2], in the presence of "free" ETC, i.e., not occupied with chlorpromazine metabolism, the process of lipid peroxidation catalyzed by reduced carriers of these chains, should proceed on them at the same rate as before. It will be recalled that the catalysis of enzymic oxidative metabolism of chlorpromazine takes place principally with the participation of ETC whose terminal component is cytochrome P-450 and not P-448 (induced by 20-methylcholanthrene) [6]. These facts suggest that two NADPH-dependent processes — oxidative conversion of chlorpromazine and POL — can take place simultaneously in the membranes of EPR, but with the participation of different ETC, competing with each other. According to the results of the present experiments, comparison of the initial velocities of enzymic POL in the control and in the presence of chlorpromazine shows that the addition of chlorpromazine leads to a reduction of the initial velocity by 30%. Besides inhibition of enzymic POL by a mechanism of competition for reduced carriers, there must therefore also exist an additional mechanism leading to total inhibition with effect from 10 min.

The products formed during NADPH-dependent oxidation of chlorpromazine, especially hydroxyl derivatives of chlorpromazine (Fig. 2), can tentatively be regarded as antioxidants. To test this hypothesis the action of certain derivatives of chlorpromazine on POL was studied

TABLE 1. Accumulation of Products Active against Thiobarbituric Acid during Enzymic and Nonenzymic POL in Rat Liver Microsomes in Presence of Chlorpromazine, Its Metabolites, and Ionol (after incubation for 30 min, in % of control)

Substance	Concentration	Enzymic POL	Nonenzymic POL
Control	—	100±9	100±6
Chlorpromazine	10^{-4} M	24±5	97±6
"	$5 \cdot 10^{-5}$ M	42±7	98±8
Nor_2-Chlorpromazine	$5 \cdot 10^{-5}$ M	46±6	93±7
SO-Chlorpromazine	$5 \cdot 10^{-5}$ M	95±8	89±10
SO-Nor_2-Chlorpromazine	$5 \cdot 10^{-5}$ M	84±11	96±8
SO-Nor_1-Chlorpromazine	$5 \cdot 10^{-}$ M	82±10	91±4
7-OH-Chlorpromazine	$5 \cdot 10^{-6}$ M	0	0
8-OH-Chlorpromazine	$5 \cdot 10^{-6}$ M	0	0
7,8-OH-Chlorpromazine	$5 \cdot 10^{-5}$ M	0	0
Ionol	$5 \cdot 10^{-6}$ M	0	0

Legend. Nor_2-Chlorpromazine — didemethylated chlorpromazine; SO-chlorpromazine — chlorpromazine sulfoxide; SO-Nor_2-chlorpromazine — didemethylated chlorpromazine sulfoxide; SO-Nor_1-chlorpromazine — monodemethylated chlorpromazine sulfoxide; 7-OH-chlorpromazine — 7-hydroxychlorpromazine; 8-OH-chlorpromazine — 8-hydroxychlorpromazine; 7,8-OH-chlorpromazine — 7,8-dihydroxychlorpromazine.

(Table 1). Whether POL was induced by an enzymic or a nonenzymic method, hydroxyl derivatives of chlorpromazine (7-OH, 8-OH, and 7,8-OH) had a powerful antioxidant action. The demethylated form of chlorpromazine (Nor_2) had an inhibitory action on enzymic POL but did not inhibit nonenzymic, ascorbate-dependent POL. This result can be explained by the formation of its hydroxy derivatives from the Nor_2 preparation in the presence of NADPH in the liver microsomes [5]. Finally, SO derivatives of chlorpromazine had no inhibitory effect on either enzymic or nonenzymic POL.

Thus in the course of NADPH-dependent metabolism of chlorpromazine derivatives with an antioxidant action are formed.

Comparison of the effectiveness of inhibition of enzymic POL by oxidative metabolites of chlorpromazine and ionol shows that complete inhibition of POL is observed with ionol in a concentration of $5 \cdot 10^{-5}$ M, but with 7-OH-chlorpromazine in a concentration of $5 \cdot 10^{-6}$ M; 7-OH-chlorpromazine is thus a more effective antioxidant than ionol.

The fact that both 7-OH-chlorpromazine and ionol completely prevent the appearance of POL products on induction with NADPH, whereas the rate of NADPH consumption is the same both in the control and with the antioxidant, will be noted.

Since hydroperoxides are formed in the reaction ($RO_2 + InH \rightarrow RO_2H + In^{\cdot}$) and the maximal ratio between NADPH, O_2 utilized, and hydroperoxides formed during enzymic oxidation of lipids is 1:4:1 [9], the total absence of POL products on the addition of ionol for 7-OH-chlorpromazine (Table 1) means that these antioxidants exhibit their inhibitory action not only through interaction with peroxide radicals, but also through interaction between antioxidants and activated forms of O_2.

The results obtained suggest that one of the mechanisms of the pharmacological action of chlorpromazine may be due to the antioxidant properties of its metabolites formed in the process of NADPH-dependent hydroxylation of chlorpromazine by monooxygenase systems.

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