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Influence of antidepressant drugs on chlorpromazine metabolism in human liver – an *in vitro* study

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Abstract:
The aim of the present study was to investigate the possible effects of antidepressant drugs (fluvoxamine, imipramine) on the metabolism of the aliphatic-type phenothiazine neuroleptic chlorpromazine in the human liver. The experiment was performed *in vitro* using human liver microsomes. The kinetic analysis of chlorpromazine metabolism carried out in the absence or presence of antidepressants showed that fluvoxamine potently inhibited chlorpromazine 5-sulfoxidation (K_i = 2.8 μM), mono-N-demethylation (K_i = 1.4 μM) and di-N-demethylation (K_i = 1.1 μM) *via* a competitive mechanism at therapeutic antidepressant concentrations. Imipramine moderately diminished the rate of chlorpromazine 5-sulfoxidation (K_i = 8.7 μM, competitive inhibition), mono-N-demethylation (Ki = 16.0 μM, non-competitive inhibition) and di-N-demethylation (K_i = 13.5 μM, mixed inhibition).
Considering the serious side-effects of chlorpromazine and some of its metabolites, metabolic interactions between this neuroleptic and antidepressant drugs (especially the chlorpromazine-fluvoxamine interaction) may be of pharmacological and clinical importance.

Key words:
imipramine, fluvoxamine, chlorpromazine metabolism, human liver microsomes, cytochrome P450, inhibition

Introduction

Joint administration of neuroleptics and tricyclic antidepressant drugs is applied in the clinic in the therapy of depression with fear, anxiety and motor excitation (agitated depression) and of delusional depression (psychotic depression), to eliminate sleep disorders in depressed subjects, in the treatment of depression in the course of schizoaffective psychoses or schizophrenia and in treatment-resistant depression [20, 24]. Moreover, selective serotonin reuptake inhibitors, which are used as antidepressants, attenuate the negative symptoms of schizophrenia [29].

Chlorpromazine is still the most widely used phenothiazine neuroleptic to treat schizophrenic patients, especially in the developing world [22]. It is a strong antagonist of the dopaminergic D_2 receptor, which is responsible for its antipsychotic effect. Chlorpromazine is also a blocker of adrenergic α_1 and muscarinic M_1 receptors, and these activities might be associated with some side-effects of the drug, such as hypotension, sedation and anticholinergic symptoms. Furthermore, it is a weak antagonist of histamine H_1 and serotonergic 5-HT_2 receptors. Apart from α_1-receptor blockade, the antihistaminic effect is also considered to be connected with the sedative side-effect of chlorpromazine [5, 17, 26].

Our recent study showed that hepatic cytochrome P450 (CYP) isoenzyme 1A2 is the only CYP isoform that catalyzes the mono-N-demethylation and di-N-demethylation of chlorpromazine and is the main isoform responsible for chlorpromazine 5-sulfoxidation at a therapeutic concentrations of the drug. Moreover, CYP3A4 contributes to a lesser degree to chlorpromazine 5-sulfoxidation [32]. It was also shown that the selective serotonin reuptake inhibitor (SSRI) fluvoxamine is a strong inhibitor of CYP1A2 and CYP2C19, while CYP1A2 and CYP3A4 are the main isoforms contributing to the N-demethylation of the tricyclic antidepressant imipramine in the human liver [4, 21, 27, 28]. Thus, metabolic interactions between chlorpromazine and fluvoxamine or imipramine seem quite possible. As shown previously, phenothiazine neuroleptics and antidepressant drugs (tricyclics, SSRIs) mutually increase their concentrations in the blood plasma of humans and in rats' plasma and brain [6–13, 25, 30, 31]. The serious consequences of the above-mentioned interactions have been observed in humans [18, 23].

The aim of the present *in vitro* study was to investigate the possible influence of fluvoxamine and imipramine on the metabolism of the aliphatic-type phenothiazine neuroleptic chlorpromazine in the human liver.

Materials and Methods

Drugs and chemicals

Chlorpromazine hydrochloride and imipramine hydrochloride were obtained from Polfa (Jelenia Góra, Poland). Fluvoxamine was provided by Duphar (Weesp, Holland). Mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine were donated by Prof. M. H. Bickel (University of Bern, Switzerland). Chlorpromazine 5-sulfoxide was synthesized in our laboratory as described previously [32]. NADPH was purchased from Sigma (St. Louis, USA). All organic solvents of HPLC purity were supplied by Merck (Darmstadt, Germany).

In vitro studies of chlorpromazine metabolism in human liver microsomes

Pooled human liver microsomes from patients HG5, HG8, HG15, HG6, HG83 and HG85 (Gentest Co., Woburn, MA, USA) were used. The optimization of chlorpromazine metabolism in human liver microsomes proceeded in three steps, during which metabolic reactions (chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation) were carried out: 1) at different time intervals, at microsomal protein and substrate concentrations of 1 mg/ml and 100 nmol/ml, respectively; 2) at different concentrations of microsomal protein, at a substrate concentration of 100 nmol/ml and an incubation time of 20 min (time interval chosen experimentally in the first step); and 3) at different substrate concentrations, at a microsomal protein concentration of 1 mg/ml (protein concentration chosen experimentally in the second step) and an incubation time of 20 min.

The influence of antidepressants on chlorpromazine metabolism *in vitro*

Basing on the obtained results, studies into chlorpromazine metabolism in human liver microsomes were carried out at the linear dependence of product formation on time, protein and substrate concentration. The rates of chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation (chlorpromazine concentrations: 5, 10, 25 and 50 μM) were assessed in the absence and presence of imipramine or fluvoxamine added *in vitro* (imipramine concentrations: 1, 2.5, 5, 10, 20 μM; fluvoxamine concentrations: 0.5, 1, 2.5, 5, 10 μM). Incubation was carried out in a system containing liver microsomes (1 mg of protein/ml), phosphate buffer (0.15 M, pH 7.4) and NADPH (1 mM). The final incubation volume was 0.5 ml. Each sample was prepared in duplicate. After a 20-min incubation, the reaction was terminated by adding 200 μl of methanol and cooling it down to 0°C.

The obtained results are presented as Dixon plots [15]. Kinetic parameters describing the chlorpromazine metabolism in human liver microsomes in the absence and presence of antidepressants were obtained using non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics).

Determination of the concentrations of chlorpromazine and its metabolites in the incubation medium

Chlorpromazine and its metabolites were analyzed using a high-performance liquid chromatography (HPLC) method as described previously [32]. Briefly, after incubation, the samples were centrifuged for 10 min at 2000 × g. The water phase containing chlorpromazine and its metabolites was extracted (pH = 12) with diethyl ether and dichloromethane (3:1, v/v). The residue obtained after evaporation of the microsomal extracts was dissolved in 100 µl of the mobile phase described below. An aliquot of 20 µl was injected into the HPLC system. The concentrations of chlorpromazine and its metabolites (chlorpromazine 5-sulfoxide, mono-N-desmethylchlorpromazine and di-N-desmethylchlorpromazine) were assayed using a LaChrom (Merck-Hitachi) HPLC system with UV detection. The analytical column (Econosphere C18, 5 µm, 4.6 × 250 mm) was purchased from Alltech (Carnforth, England). The mobile phase consisted of acetate buffer, pH = 3.4 (100 mmol ammonium acetate, 20 mmol citric acid, and 1 ml triethylamine in 1000 ml of the buffer adjusted to pH = 3.4 with 85% phosphoric acid), and acetonitrile at a proportion of 1:1. The flow rate was 0.6 ml/min, and the column temperature was 30°C. The absorbances of chlorpromazine and its metabolites were measured at a wavelength of 254 nm. The compounds were eluted in the following order: chlorpromazine 5-sulfoxide (10.31 min), di-N-desmethylchlorpromazine (13.31 min), chlorpromazine (15.41 min) and mono-N-desmethylchlorpromazine (16.81 min). The sensitivity of the HPLC method allowed the quantification of chlorpromazine concentrations as low as 0.001 nmol/ml, chlorpromazine 5-sulfoxide concentrations as low as 0.004 nmol/ml, di-N-desmethylchlorpromazine concentrations as low as 0.002 nmol/ml and mono-N-desmethylchlorpromazine concentrations as low as 0.004 nmol/ ml in the microsomal suspension. The accuracy of the method was estimated to be 2%. The intra- and inter-assay coefficients of variance were below 3% and 2%, respectively.

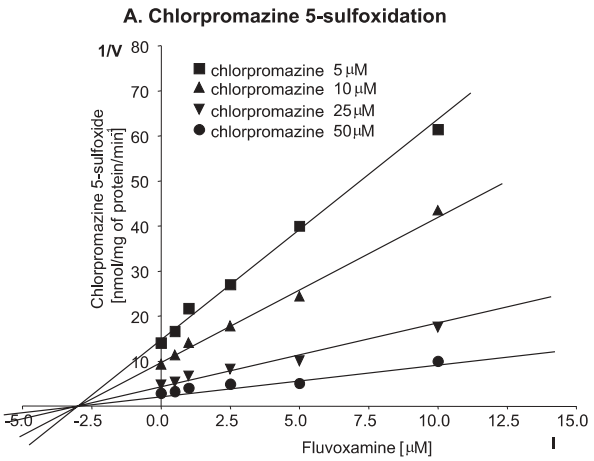
Results

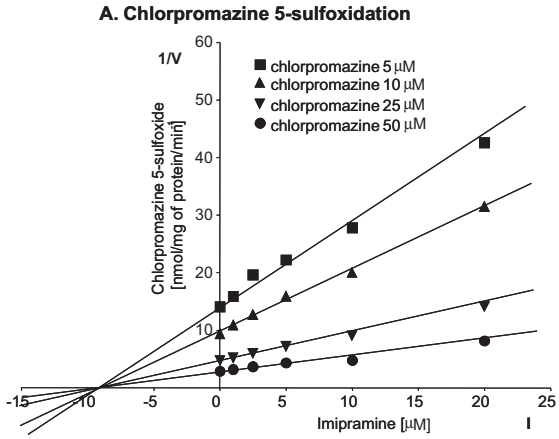
Under the experimental conditions, chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation production proceeded linearly up to 45 min, and over the entire range of tested protein concentrations (up to 2 mg/ml). Moreover, the amount of chlorpromazine metabolites formed significantly increased up to 150 µM of the substrate concentration in the case of 5-sulfoxidation and di-N-demethylation and up to 75 µM in the case of mono-N-demethylation (data not shown). The substrate con-

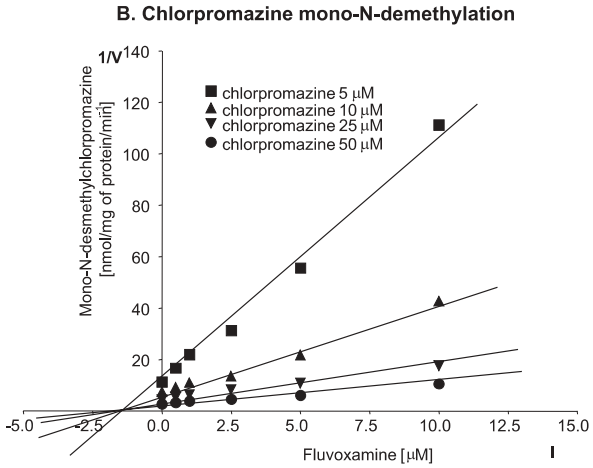
Tab. 1. The influence of fluvoxamine on the metabolism of chlorpromazine *in vitro* in human liver microsomes

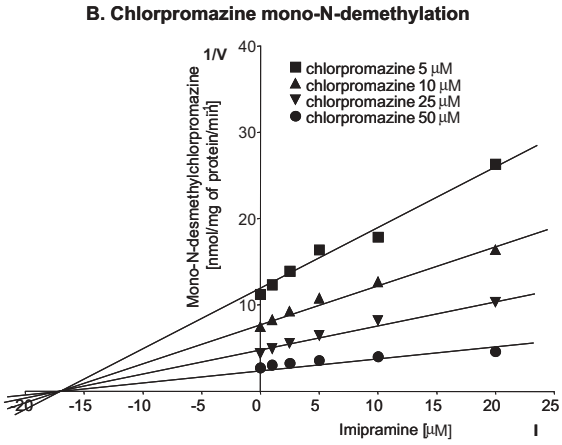
Inhibitor (fluvoxamine) concentrations [µM]	Kinetic parameters of chlorpromazine metabolism								
	Chlorpromazine 5-sulfoxidation			Chlorpromazine mono-N-demethylation			Chlorpromazine di-N-demethylation		
	K_m [µM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)	K_m [µM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)	K_m [µM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)
0 (control)	42.14	0.61	0.014	24.20	0.49	0.020	17.67	0.20	0.011
0.5	56.33	0.63	0.011	41.34	0.56	0.013	18.21	0.17	0.009
1.0	57.80	0.52	0.009	50.45	0.53	0.010	33.45	0.20	0.006
2.5	67.32	0.47	0.007	88.96	0.60	0.007	51.92	0.20	0.004
5.0	71.15	0.38	0.005	289.30	1.11	0.004	89.35	0.20	0.002
10.0	111.80	0.32	0.003	1700.00	3.93	0.002	40.91	0.05	0.001

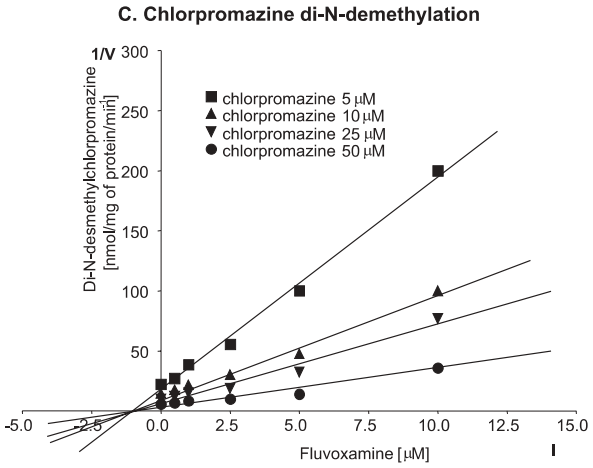
The presented values of the Michaelis-Menten constants (K_m), the maximum velocities of the reactions (V_{max}) and the intrinsic clearances (CL) for the specific metabolic pathway were obtained using non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics). The values of the inhibition constants (K_i) are presented in Table 3











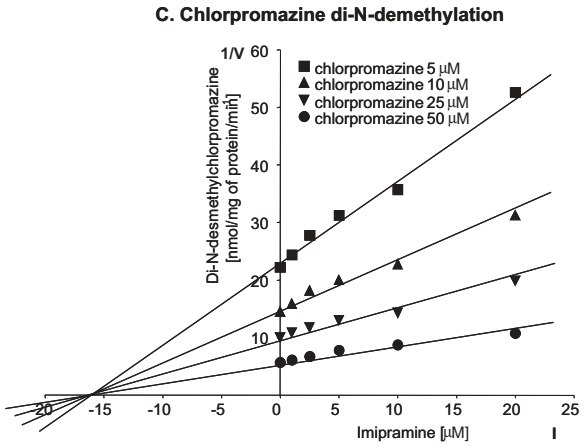


Fig. 1. The influence of fluvoxamine on the metabolism of chlorpromazine in human liver microsomes: (**A**) chlorpromazine 5-sulfoxidation; (**B**) chlorpromazine mono-N-demethylation; and (**C**). chlorpromazine di-N-demethylation (Dixon's plots). Each point represents the mean value of two independent analyses. K_i values are shown in Table 3. V: velocity of the reaction; I: concentration of the inhibitor (fluvoxamine)

Fig. 2. The influence of imipramine on the metabolism of chlorpromazine in human liver microsomes: (**A**) chlorpromazine 5-sulfoxidation; (**B**) chlorpromazine mono-N-demethylation; and (**C**). chlorpromazine di-N-demethylation (Dixon's plots). Each point represents the mean value of two independent analyses. K_i values are shown in Table 3. V: velocity of the reaction; I: concentration of the inhibitor (imipramine)

Tab. 2. The influence of imipramine on the metabolism of chlorpromazine *in vitro* in human liver microsomes

Inhibitor (imipramine) concentrations [μM]	Kinetic parameters of chlorpromazine metabolism								
	Chlorpromazine 5-sulfoxidation			Chlorpromazine mono-N-demethylation			Chlorpromazine di-N-demethylation		
	K_m [μM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)	K_m [μM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)	K_m [μM]	V_{max} [nmol/mg protein/min]	CL (V_{max}/K_m)
0 (control)	42.14	0.61	0.014	24.20	0.49	0.020	17.67	0.20	0.011
1.0	53.84	0.64	0.012	23.11	0.43	0.019	18.29	0.19	0.010
2.5	59.69	0.59	0.010	24.23	0.40	0.016	20.53	0.18	0.009
5.0	70.17	0.55	0.008	26.59	0.37	0.014	19.53	0.16	0.008
10.0	83.11	0.52	0.006	22.63	0.30	0.013	21.08	0.14	0.007
20.0	76.38	0.30	0.004	35.89	0.31	0.009	29.91	0.13	0.004

The presented values of the Michaelis-Menten constants (K_m), the maximum velocities of the reactions (V_{max}) and the intrinsic clearances (CL) for the specific metabolic pathway were obtained using non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics). The values of inhibition constants (K_i) are presented in Table 3

Tab. 3. The potency of fluvoxamine and imipramine to inhibit chlorpromazine metabolism *in vitro* in human liver microsomes

Inhibitor	Inhibition of chlorpromazine metabolism K_i (μM) and type of inhibition*		
	Chlorpromazine 5-sulfoxidation	Chlorpromazine mono-N-demethylation	Chlorpromazine di-N-demethylation
Fluvoxamine	2.81 (competitive)	1.43 (competitive)	1.14 (competitive)
Imipramine	8.66 (competitive)	15.95 (non-competitive)	13.50 (mixed)

The presented inhibition constants (K_i) for the inhibition of a specific metabolic pathway were obtained using a non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics). * The mechanisms of inhibition are valid for fluvoxamine concentrations up to 2.5 μM and for imipramine concentrations up to 10 μM

sumption was 4–9%. Considering the above observations, the following parameters were chosen for further studies: an incubation time of 20 min, a microsomal protein concentration of 1 mg/ml and substrate concentrations of 5, 10, 25 and 50 μM. The first two concentrations of the substrate were in the range of pharmacological neuroleptic concentrations present *in vivo*, while the last two concentrations were close to the control values of K_m obtained *in vitro* for the metabolism of chlorpromazine (Tab. 1).

The incubation of liver microsomes with chlorpromazine (in the absence and presence of inhibitor) was carried out in linear reaction conditions with respect to time, microsomal protein and substrate concentration. The Dixon plots of chlorpromazine metabolism carried out in human liver microsomes, in the absence or presence of antidepressants, showed that fluvoxamine potently inhibited chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation, while imipramine moderately diminished the rate of chlorpromazine metabolism (Figs. 1 and 2). Kinetic parameters describing chlorpromazine metabolism in human liver microsomes (K_m, V_{max}, CL), in the absence and presence of antidepressants, obtained using non-linear regression analysis (Program Sigma Plot 8.0; Enzyme Kinetics), are presented in Tables 1 and 2.

Fluvoxamine inhibited chlorpromazine 5-sulfoxidation (K_i = 2.81 μM), mono-N-demethylation (K_i = 1.43 μM) and di-N-demethylation (K_i = 1.14 μM) (Tabs. 1 and 3) *via* a competitive mechanism. However, at higher fluvoxamine concentrations (5–10 μM) chlorpromazine 5-sulfoxidation and di-N-demethylation were inhib-

ited *via* a mixed mechanism, while in the case of mono-N-demethylation, an additional allosteric effect (causing an increase in V_{max}) seemed to appear. On the other hand, imipramine diminished the rate of chlorpromazine 5-sulfoxidation in a competitive manner (K_i = 8.66 μM), of mono-N-demethylation in a non-competitive manner (K_i = 15.95 μM) and of di-N-demethylation (K_i = 13.5 μM) in a mixed-type manner (Tabs. 2 and 3). At a higher imipramine concentration (20 μM), the inhibitions of chlorpromazine 5-sulfoxidation and mono-N-demethylation were also of mixed character. In all cases, the value of clearance (CL) was decreased in the presence of an antidepressant.

Discussion

The obtained results suggest that fluvoxamine or imipramine used at therapeutic concentrations exert an inhibitory effect on chlorpromazine metabolism in the human liver, although their potencies toward particular metabolic pathways are different. The kinetic analysis of chlorpromazine metabolism, carried out using human liver microsomes, showed that fluvoxamine potently decreased the rates of chlorpromazine 5-sulfoxidation, mono-N-demethylation and di-N-demethylation (K_i = 2.8, 1.4 and 1.1 μM, respectively). Regarding the above results and the previous experimental data (showing that CYP1A2 is the main CYP isoform catalyzing the metabolism of chlorpromazine, while fluvoxamine is a potent inhibitor of CYP1A2 [4, 27, 32]), it seems clear that CYP1A2 is chiefly involved in the metabolic interactions between chlorpromazine and fluvoxamine observed in the present study. The moderate inhibition of chlorpromazine 5-sulfoxidation (K_i = 8.7 μM), mono-N-demethylation (K_i = 15.9 μM) and di-N-demethylation (K_i = 13.5 μM) by imipramine may stem from the competition for the active sites of CYP1A2 and CYP3A4, which are mainly involved in chlorpromazine 5-sulfoxidation and imipramine N-demethylation, as well as from the ability of imipramine to form reactive metabolites [7, 16, 21, 32].

Although the therapeutic plasma concentrations of chlorpromazine, fluvoxamine and imipramine reach to 1 μM, their concentrations in the liver may be about 10 times higher (up to 10 μM) than in the plasma owing to drug distribution [2, 14, 33–35]. Hence, the interaction between chlorpromazine and antidepressants observed in the present study *in vitro* (especially the chlorpromazine-fluvoxamine interaction) should also be observed *in vivo*, since the calculated K_i values for the tested antidepressants are below (the fluvoxamine-chlorpromazine interaction) or close (the imipramine-chlorpromazine interaction) to the presumed concentration range for the above antidepressants in the liver *in vivo*, based on both pharmacological experiments and data from psychiatric patients. Accordingly, a study by Loga et al. [23] showed a significant elevation of the plasma chlorpromazine concentration after concomitant tricyclic antidepressant amitriptyline administration to schizophrenic patients. That increase was accompanied by further amitriptyline-induced potentiation of diminished blood pressure, which is observed in patients treated with chlorpromazine. It is not unlikely that metabolic interactions between chlorpromazine and the tested antidepressants may also involve CYP2D6, which is the main isoenzyme responsible for chlorpromazine 7-hydroxylation [36]. As shown previously, imipramine exerts a relatively potent inhibitory effect on human CYP2D6 (K_i = 4 μM), while fluvoxamine has been reported to be a less potent inhibitor of that isoenzyme (K_i = 10 μM) [1, 3, 7, 19].

Considering the strong receptor blocking properties of chlorpromazine (D_2, α_1 and M_1 receptors), which lead to its serious side-effects (a decrease in blood pressure, anticholinergic effects, extrapyramidal symptoms), chlorpromazine-fluvoxamine and chlorpromazine-imipramine interactions may be of pharmacological and clinical importance. Moreover, it must not be forgotten that, simultaneously with the elevation of the chlorpromazine concentration, an increase in the antidepressant concentration may occur. The latter aspect of the interaction should also be considered in clinical practice, in particular that of tricyclic antidepressants (e.g., imipramine) and phenothiazine neuroleptics that block α_1, M_1 and H_1 receptors and cause similar side-effects (hypotension, sedation, anticholinergic symptoms).

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