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Artifacts in the analysis of thioridazine and other neuroleptics

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Abstract: Although the sensitivity to light of thioridazine and its metabolites has been described, the problem does not seem to be widely acknowledged. Indeed, a survey of the literature shows that assays of these compounds under light-protected conditions have been performed only in a few of the numerous analytical studies on this drug. In the present study, thioridazine, its metabolites, and 18 other neuroleptics were tested for their sensitivity to light under conditions used for their analysis. The results show that light significantly affects the analysis of thioridazine and its metabolites. It readily causes the racemization of the isomeric pairs of thioridazine 5-sulphoxide and greatly decreases the concentration of thioridazine. This sensitivity to light varied with the medium used (most sensitive in acidic media) and also with the molecule (in order of decreasing sensitivity: thioridazine > mesoridazine > sulforidazine). Degradation in neutral or basic media was slow, with the exception of mesoridazine in a neutral medium. Twelve other phenothiazines tested, as well as chlorprotixene, a thioxanthene drug, were found to be sensitive to light in acidic media, whereas flupenthixol and zuclopenthixol (two thioxanthenes), clozapine, fluperlapine, and haloperidol (a butyrophenone) did not seem to be affected. In addition to being sensitive to light, some compounds may be readily oxidized by peroxide-containing solvents.

Keywords: *Thioridazine; butyrophenones; phenothiazines; thioxanthenes; neuroleptics; therapeutic drug monitoring; light; artifacts.*

Introduction

Thioridazine (THD), a phenothiazine neuroleptic drug, is commonly prescribed for schizophrenia and other psychiatric disorders. It is extensively biotransformed by N-demethylation into northioridazine (*N*-THD), by side-chain oxidation into mesoridazine (THD 2-SO) and sulforidazine (THD 2-SO_2), and by ring sulphoxidation into thioridazine 5-sulphoxide (THD 5-SO) [1–3]. This last compound exists as two diastereoisomeric pairs, called 'THD 5-SO fast eluting (FE)' and 'THD 5-SO slow eluting (SE)' owing to the properties they exhibit when separated by different chromatographic methods [4, 5]. Although published studies do not suggest a strong relationship between blood levels and therapeutic effects of antipsychotic drugs, some authors claim that it is useful to monitor the concentrations of thioridazine and/or its metabolites [6–9]. Indeed, patients in whom the highest plasma drug levels are achieved do not usually have the best clinical outcome [10, 11].

The light-induced racemization of the diastereoisomeric pairs of thioridazine 5-sulphoxide, which has been recently described, could greatly influence the analysis of these compounds [12]. Since then, all analyses of THD and its metabolites have been performed in our laboratory under light-protected conditions, but it was not clear whether thioridazine, mesoridazine and sulforidazine were also sensitive to light and to what extent. Indeed, several papers have described phenomena such as the photolysis of some chlorine-containing drugs or photodegradation of phenothiazines and their subsequent covalent binding to various macromolecules [13–17]. Nevertheless, in only a few studies have thioridazine and its metabolites been analysed under light-protected conditions [10, 18, 19]; many other studies have made no reference to such special precautions [2, 5, 20–36] possibly because, at least under some conditions, concentrations of THD, mesoridazine and sulforidazine seem to be unaffected by light. Thus, in control experiments during the study on the light-induced racemization of the isomeric pairs of THD 5-SO, concentrations of thioridazine, mesoridazine and sulforidazine diluted in an HPLC solvent (hexane–methylene chloride–methanol; 8.3:1:0.7, v/v/v) were not modified by exposure to a UV lamp for 1 h [12].

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The purpose of the present study was to investigate the sensitivity to light of thioridazine and its metabolites under conditions used for their analysis. The experiments were subsequently extended to 18 other neuroleptics, mainly of the phenothiazine and thioxanthene types.

Materials and Methods

Reagents

Pipothiazine, thioridazine and its metabolites were obtained as previously described [12]. Chlorpromazine was kindly provided by Rhône–Poulenc (Paris, France), and chlorprothixene, flupenthixol and zuclopenthixol by Lundbeck (Copenhagen, Denmark). Other neuroleptics were obtained from commercial sources. All other reagents used were of analytical or HPLC grade.

Analysis under light-protected or light-exposed conditions of samples of blank plasma containing thioridazine and metabolites

One-ml portions of blank plasma were spiked with known amounts of THD and its metabolites (see Table 1), and extracted and analysed as previously described [12]. To 1 ml of heparinized plasma in a tube were added 500 ng of pipothiazine (the internal standard), 400 μl of 2 M sodium hydroxide, and 4 ml of diethyl ether–hexane (3:1, v/v); the extraction was performed in a mechanical shaker for 20 min. After centrifugation (5 min, 2800*g*), the organic layer was transferred to a tube containing 1.2 ml of 0.1 M hydrochloric acid. After 15 min of shaking and centrifugation, the organic phase was discarded; the acid phase was transferred to a tube containing 200 μl of 2 M sodium hydroxide and was extracted with two 2-ml portions of diethyl ether–hexane. The organic phase was then evaporated under nitrogen at 40°C; the residue was dissolved in 500 μl of hexane–methylene chloride–methanol (8.3:1:0.7, v/v/v), and 100 μl was injected into the liquid chromatograph. For the chromatographic separations, a Waters Z-module radial compression separation system (Waters, Kloten, Switzerland) was used with a cartridge (8 mm i.d.) packed with 5-μM microparticulate silica gel and a guard column filled with the same material. The mobile phase was 2,2,4-trimethylpentane–methylene chloride–methanol (8.3:1:0.7). The flow rate was set at 2.25 ml min^{-1}, and the detector at 254 nm. Throughout the analysis, light-protected and light-exposed samples were kept in aluminium-wrapped foil and in bare Pyrex tubes, respectively. The samples were always manipulated alternately in a room with dim neon light and with the window-blinds closed. The only difference between the control and the light-exposed samples was that the latter were exposed to indirect sunlight (near the windows in a room with the window-blinds open) for about 20 min twice during the shaking steps of the extraction procedure.

Stability of thioridazine and its metabolites in different media

The drugs were dissolved in 5-ml portions of different buffers or solvents (phosphate buffer 0.1 M Na_2HPO_4–NaH_2PO_4, pH 7.0, and carbonate buffer, 0.1 M Na_2CO_3–$NaHCO_3$, pH 9.4) at a concentration of 5 μg ml^{-1} for each compound; the solutions were placed in the dark or exposed to indirect sunlight (near the windows). At different times, 300 μl (to completely flush the 100-μl injection loop) was injected directly into the chromatograph.

As the normal-phase column used for the first experiment was not compatible with aqueous media, another method was chosen [37]. A 300-mm Nucleosil 10 CN column (4 mm i.d.) (Macherey–Nagel, Oensingen, Switzerland) was used. The mobile phase was acetonitrile–methanol–phosphate buffer (0.01 M Na_2HPO_4 adjusted to pH 6.6 with 1 M H_3PO_4) (3:4.5:2.5, v/v/v). The flow rate was set at 1.5 ml min^{-1}.

Stability of other neuroleptics in acidic media

Eighteen other neuroleptics, mainly of the phenothiazine and the thioxanthene types, were dissolved in 0.1 M HCl at a concentration of 5 μg ml^{-1} and placed in the dark or exposed to indirect sunlight (near the windows). At 0 and 1 h, 300 μl was directly injected into the HPLC.

Results

Analysis of plasma containing thioridazine and its metabolites under light-exposed and light-protected conditions

Table 1 shows typical results obtained after extraction and analysis of samples of blank plasma containing various concentrations of THD and its metabolites (samples A and B differed only in the presence of added THD 5-

Table 1
Drug concentrations (mean ± SD in ng ml^{-1}, RSD in brackets) and mean chromatographic peak areas (arbitrary units) measured in two blank plasma samples (A and B) after extraction with and without light-protection

			Thioridazine	Mesoridazine	Sulforidazine	THD 5-SO (FE)	THD 5-SO (SE)	Pipothiazine
Plasma A		theoretical concentrations	500	500	250	1000	0	—
	dark	measured concentrations	499 ± 23 (5%)	472 ± 18 (4%)	251 ± 9 (3%)	972 ± 7 (1%)	6 ± 1 (22%)	—
		measured areas	3.7	3.08	1.33	2.89	0.01	4
	light	measured concentrations	388 ± 25 (6%)	1463 ± 134 (9%)	1034 ± 116 (11%)	877 ± 48 (5%)	848 ± 80 (9%)	—
		measured areas	0.66	2.39	1.34	0.61	0.7	0.91
Plasma B		theoretical concentrations	500	500	250	0	1000	—
	dark	measured concentrations	455 ± 14 (3%)	503 ± 31 (6%)	245 ± 3 (1%)	62 ± 8 (13%)	1131 ± 106 (9%)	—
		measured areas	2.64	2.63	1.03	0.13	3.01	3.19
	light	measured concentrations	416 ± 46 (11%)	1007 ± 115 (11%)	573 ± 64 (11%)	898 ± 76 (8%)	955 ± 102 (11%)	—
		measured areas	1.19	2.84	1.23	1.07	1.27	1.41

The plasma samples were spiked with various quantities of thioridazine and metabolites (theoretical concentrations are given). The values are the mean of four experiments. Pipothiazine is the internal standard.
THD 5-SO (FE): thioridazine 5-sulphoxide fast-eluting; THD 5-SO (SE): thioridazine 5-sulphoxide slow-eluting.

SO (FE) and THD 5-SO (SE), respectively), under light-protected and light-exposed conditions. Mean values close to the theoretical concentrations and with small standard deviations were obtained for all compounds when extraction was performed with light protection. Otherwise, the results were poor; complete racemization of the isomeric pairs of THD 5-SO occurred, and mean values obtained for the other compounds differed markedly from the theoretical concentrations. These results are explained by strong reductions in the chromatographic peak areas of at least thioridazine and pipothiazine (the internal standard).

Stability of thioridazine and its metabolites

Figure 1 shows the kinetics of disappearance of standard solutions of thioridazine, mesoridazine, sulforidazine and pipothiazine in 0.1 M HCl and exposed to indirect sunlight, in contrast to the stability of these compounds when stored in the dark. It is of interest that these kinetics vary with the molecule, the light-induced decrease being very fast for thioridazine and pipothiazine but almost unnoticeable for sulforidazine, which confirms results obtained in the first experiment. Figure 2 shows typical HPLC chromatograms of a degraded and an undegraded sample of thioridazine and pipothiazine, obtained during the kinetic experiments.

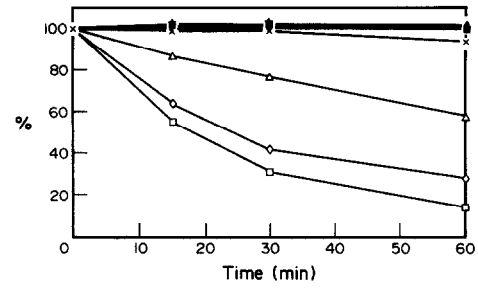

Figure 1
Disappearance kinetics of thioridazine (□), mesoridazine (△), sulforidazine (×) and pipothiazine (◇) in 0.1 M HCl and stored either in the dark (in black) or exposed to light (in white). The values are given as the relative areas (%) of the peaks measured at different times divided by the areas of the peaks measured at time 0 h. The results are the mean of two experiments.

Table 2 shows the stability of the four compounds in a 0.1 M phosphate buffer (pH 7.0) or in a 0.1 M carbonate buffer (pH 9.4), in the dark or in indirect sunlight. These results show that in a neutral medium only mesoridazine is strongly affected by light whereas under alkaline conditions all the drugs are only slightly affected.

Finally, the stability of thioridazine in the presence of some solvents used during the extraction steps (hexane, diethyl ether and methanol) in acidic media and in the dark, was tested. In accordance with previously published results [2], a decrease in the THD

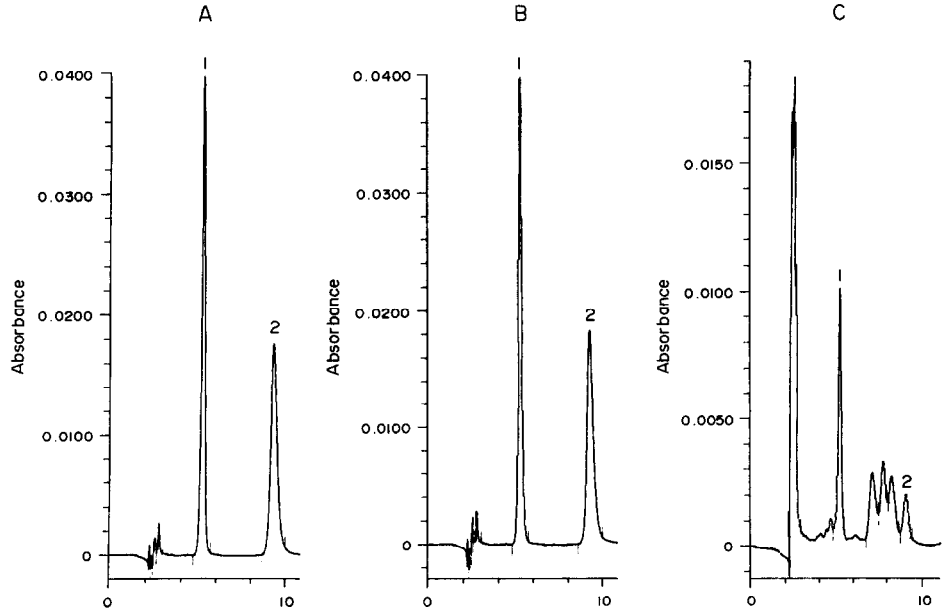

Figure 2
High-performance liquid chromatograms obtained after injection of 0.1 M HCl containing thioridazine (peak 2, 9.01 min), and pipothiazine (peak 1, 5.12 min) at time 0 (A), stored in the dark for 60 min (B) or exposed to light for 60 min (C).

Table 2
Relative peak areas (%) of thioridazine, mesoridazine, sulforidazine and pipothiazine after 1 h compared with those at time 0 h

	pH 7.0		pH 9.4	
	Dark	Light	Dark	Light
Thioridazine	101	93	101	88
Mesoridazine	103	64	103	86
Sulforidazine	103	107	107	90
Pipothiazine	100	96	107	96

The substances were either diluted in 0.1 M phosphate buffer (pH 7.0) or in 0.1 M carbonate buffer (pH 9.4) and stored in the dark or exposed to light. The results are the mean of two experiments.

concentration was observed with peroxide-containing solvents (data not shown). Surprisingly there was less peroxide in diethyl ether than in methanol or hexane.

Stability of other neuroleptics in acidic media

Table 3 lists the relative peak areas (%) representing the relative concentrations of various neuroleptics in 0.1 M HCl after exposure to indirect sunlight for 1 h compared with those at time 0 h. No decrease in the original concentration was observed when these samples were stored in the dark (data not shown). These results show that all the phenothiazines tested are light-sensitive when stored under acidic conditions; fluphenazine is the most sensitive (only 3% of the original concentration was found after 1 h). All other drugs tested were stable in these conditions, with the exception of chlorprothixene.

Table 3
Relative peak areas (%) of various neuroleptics in 0.1 N HCl after 1 h exposure to indirect sunlight compared with those at time 0 h

Phenothiazines	
Chlorpromazine	70
Fluphenazine	3
Levomepromazine	51
Periciazine	79
Perphenazine	43
Promazine	59
Promethazine	52
Thiethylperazine	62
Thiopropazate	49
Thioproperazine	20
Trifluoperazine	71
Triflupromazine	58
Thioxanthenes	
Chlorprothixene	57
Flupenthixol	98
Zuclopenthixol	98
Other drugs	
Clozapine	100
Fluperlapine	100
Haloperidol	99

The results are the mean of two experiments.

Discussion

Photolysis of some chlorine-containing drugs and photodegradation of phenothiazines and their covalent binding to various macromolecules are well-known phenomena [13–17] but in only a few studies on the analysis of thioridazine and its metabolites [10, 18, 19] were solutions protected from light; many reports make no mention of such special precautions [2, 5, 20–36]. The reason may be that, at least in some instances, THD, mesoridazine and sulforidazine seem to be unaffected by light [12].

In the present study, extraction of two plasma samples containing various concentrations of THD and its metabolites, with and without light-protection, showed clearly that even indirect sunlight can significantly affect the results. The most sensitive compounds seemed to be the isomeric pairs of THD 5-SO, which readily racemized; this finding confirmed previously published results [12]. Marked reductions in the chromatographic peak areas of thioridazine and pipothiazine were also observed. Unlike the findings reported on the photo-induced binding of phenothiazines to macromolecules [14–17], the analysis of protein-free samples compared with that of protein-containing samples, under dark and light conditions (indirect sunlight), yielded no evidence of the covalent binding of thioridazine and metabolites to plasma proteins (data not shown). This difference may be due to the use in the earlier studies [14–17] of UV irradiation corresponding to the wavelength of maximum absorbance of the phenothiazine.

Different media used during the extraction procedure were then tested; pipothiazine, THD and its metabolites were shown to be particularly sensitive to the effects of light when diluted in an acidic medium. When THD was tested (Fig. 2), two of the additional peaks which appeared on the chromatogram corresponded to the isomeric pairs of THD 5-SO. This result shows that some oxidation had occurred, a phenomenon that was found also in the presence of some peroxide-containing

solvents. It is of interest that the compounds tested were not equally sensitive to light; thioridazine was the most sensitive, followed by pipothiazine, mesoridazine and sulforidazine. It was also clear that the kinetics of degradation were very fast in acidic media; less than half of the original concentration of thioridazine and pipothiazine was present after only 30 min (Fig. 1). Light-induced degradation of these compounds occurred also in neutral or basic media (Table 2) although at a slower rate, with the exception of mesoridazine, which showed considerable degradation at pH 7.0.

The results with thioridazine and pipothiazine, both phenothiazines, prompted further tests on the stability of several other neuroleptics in acidic media when exposed to light. These neuroleptics were mainly phenothiazines and thioxanthenes, two chemically similar classes of drugs. The results (Table 3) showed that all phenothiazines tested were sensitive to light and that this effect was particularly spectacular in the case of fluphenazine; only 3% of the original concentration of this drug was found after 1 h exposure to light. One thioxanthene (chlorprothixene) of the three tested was found to be light-sensitive whereas the others, flupenthixol and zuclopenthixol, as well as clozapine, fluperlapine and haloperidol (a butyrophenone), seemed to be unaffected by light.

It may be worth pointing out that indirect sunlight was used in these experiments instead of a standard source of light, such as a UV lamp, in order to simulate everyday laboratory practice. The results, which were dependent on the amount and probably also on the quality of light, varied with the weather and with the location of the extraction apparatus (near the windows or not) and are, therefore, only indicative. Consequently, analyses in different laboratories will not be equally sensitive to light; where analytical methods that do not include an acidic step [10, 19, 27, 28, 30, 32, 36] during the extraction procedure are used, fewer light-sensitivity problems would be expected to occur although, as shown in this work, some compounds such as mesoridazine are sensitive even in neutral media.

In conclusion, although the effects of exposure to light during the analysis of phenothiazines and thioxanthenes may vary between laboratories, the present study shows that in general all manipulations of samples containing these compounds should be performed protected from light. Finally, all solvents should be tested for the absence of peroxides, divided into aliquots and stored in separate darkened vessels.

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