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PHENOTHIAZINE PHOTOTOXICITY: AN EXPERIMENTAL STUDY ON CHLORPROMAZINE AND RELATED TRICYCLIC DRUGS

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Abstract. A large number of phenothiazines and chemically related tricyclic drugs have been studied with respect to their phototoxic potency. Two methods were used, an in vivo technique based on the inflammatory response of the mouse tail after systemic administration of the drug plus UVA irradiation, and an in vitro method based on growth inhibition of *Candida albicans*. Of 27 commercial tricyclic drugs tested in vivo, the most potent were chlorpromazine and two other chlorinated compounds, prochlorperazine and perphenazine. Tricyclic drugs lacking nitrogen, sulphur, or both in their ring system showed no activity. All compounds phototoxic in the mouse were so in the yeast test as well. Here, however, the thioxanthenes (lacking nitrogen) were also highly active.

Key words: Ultraviolet light; Phototoxicity; Phenothiazines; Chlorpromazine; Tricyclic drugs

Chlorpromazine is capable of inducing phototoxic as well as photoallergic skin reactions, as has been widely documented in clinical and experimental studies. Other phenothiazines too may enhance light sensitivity, but it is not known why chlorpromazine is the most potent phototoxic agent in this drug group. Thus, the molecular characteristics attributable to phototoxic activity have not been elucidated. It was therefore considered essential to investigate a large number of phenothiazines and chemically related tricyclic compounds. An in vivo as well as an in vitro method was used for this quantitative study on drug phototoxicity.

MATERIAL AND METHODS

The in vivo and in vitro methods used for the study of drug phototoxicity have been described recently (6, 7). In the in vivo test albino mice were injected intraperitoneally with the test drug. The dose range was from 2.5 mg/kg up to a reacting dose or a lethal one, usually around 80 mg/kg. Poorly soluble compounds (periciazine, methdilazine and acetophenazine) were converted to their more soluble hydrochlorides with 0.1 N hydrochloric acid. The practically insoluble phenothiazine was administered suspended in aqueous methyl cellulose solution via a gastric tube (control animals received the vehicle only). The tails of the animals were immediately exposed to longwave ultraviolet light (UVA) for 5 hours, the measured average intensity of radiation being 5.0×10^4 erg/cm^2 sec. The degree of phototoxic inflammation was calculated on the basis of the increase in wet weight of the mouse tail. In vitro, the capacity of different drugs to inhibit the out-growth of *Candida albicans* exposed to UVA in culture was studied.

In both techniques chlorpromazine (CPZ) was chosen as the reference compound. The phototoxic index (PI) for various drugs in the mouse tail method was obtained by dividing the minimum phototoxic dose (mmol/kg) by that of CPZ, which latter was given the PI 10.0. The minimum phototoxic dose was the lowest one capable of inducing a tissue wet weight increase differing significantly from controls ($p<0.05$). Similarly, a drug PI in the *Candida albicans* method was calculated on the basis of the lowest concentration giving a yeast-free zone of 15 mm diameter; the exact concentration was obtained by extrapolating from a dose–response curve. The PI was given as 0 when no yeast inhibition was observed with at least a 10% drug in ethanol solution. NT=not tested.

A total of 27 commercially available phenothiazines and related tricyclic drugs were studied, in most cases with both methods. For statistical evaluation, the Student's *t*-test was used throughout.

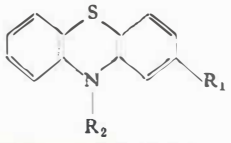
RESULTS AND COMMENTS

Mouse tail method

The mouse tail technique for acute drug phototoxicity, developed with CPZ as a test compound (7), has been found in the present work to be applicable for other phenothiazines too. It has thereby been possible to elucidate the relationship between molecular structure and phototoxic activity within this group of tricyclic compounds.

The phototoxic capacity of the drugs tested as PI

Table I. *Phototoxic index in vivo and in vitro for various phenothiazines*

Phenothiazine derivatives: 

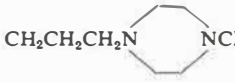
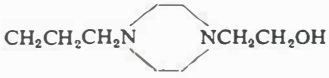
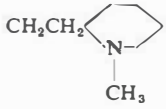
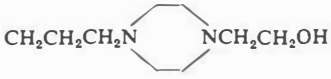
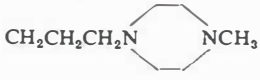
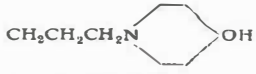
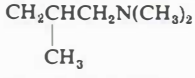
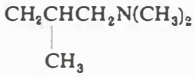
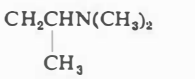
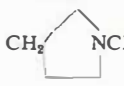
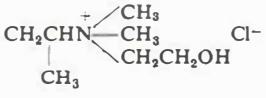
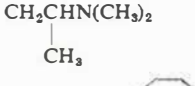
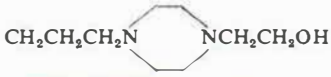
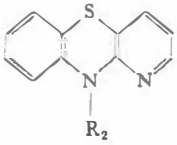
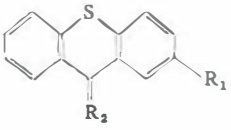
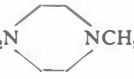
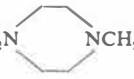
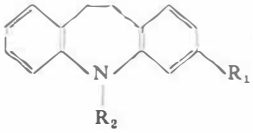
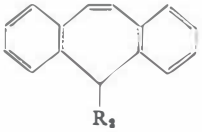
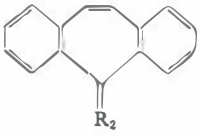
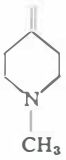
Compound				PI	PI
Generic name	Trade name	R_1	R_2	Mouse	Candida
1. Chlorpromazine	Hibernal® Klorpromex® Largactil®	Cl	$CH_2CH_2CH_2N(CH_3)_2$	10	1.0
2. Prochlorperazine	Stemetil®	Cl		6.6	1.3
3. Perphenazine	Trilafon®	Cl		6.2	0.9
4. Thioridazine	Mallorol®	SCH_3		2.9	0.6
5. Fluphenazine	Pacinol® Siqualone®	CF_3		1.7	0.9
6. Acetylpromazine	Plegicil (vet.)®	$COCH_3$	$CH_2CH_2CH_2N(CH_3)_2$	1.2	NT
7. Trifluoperazine	Terfluzin®	CF_3		0.8	1.9
8. Periciazine	Neulactil®	CN		0.7	NT
9. Promazine	Protactyl®	—	$CH_2CH_2CH_2N(CH_3)_2$	0.6	3.0
10. Levomepromazine	Nozinan®	OCH_3		0.6	0.7
11. Alimemazine	Theralen®	—		0.4	0.9
12. Promethazine	Lergigan®	—		0.3	4.6
13. Methdilazine	Tacaryl®	—		0.3	1.7
14. Phenothiazine	Hippozin (vet.)®	—	—	0.2	NT
15. *N*-hydroxyethyl-promethazinechloride	Aprobit®	—		0	0.6
16. Propiomazine	Propavan®	$COCH_2CH_3$		0	0
17. Acetophenazine	Tindala®	$COCH_3$		0	NT

Table II. *Phototoxic index in vivo and in vitro for various tricyclic compounds, related to chlorpromazine*

Compound					PI	PI
Generic name	Trade name	Structures	R_1	R_2	Mouse	Candida
18. Protipendyl			—	$-CH_2CH_2CH_2N(CH_3)_2$	1.1	0.2
Thioxanthenes						
19. Chlorprotixene	Truxal®		Cl	$=CHCH_2CH_2N(CH_3)_2$	0	27.6
20. Clopentixol	Sordinol®		Cl	$=CHCH_2CH_2N$  NCH_2CH_2OH	0	24.3
21. Flupentixol	Fluanxol®		CF_3	$=CHCH_2CH_2N$  NCH_2CH_2OH	0	7.5
Azepines						
22. Imipramine	Tofranil®		—	$-CH_2CH_2CH_2N(CH_3)_2$	0	0
23. Clomipramine	Anafranil®		Cl	$-CH_2CH_2CH_2N(CH_3)_2$	0	NT
24. Trimipramine	Surmontil®		—	$-CH_2CHCH_2N(CH_3)_2$ CH_3	0	0
Cycloheptenes						
25. Amitriptyline	Larozyl® Saroten® Tryptizol®		—	$=CHCH_2CH_2CH_2N(CH_3)_2$	0	0
26. Protriptyline	Concordin®		—	$=CH_2CH_2CH_2NHCH_3$	0	NT
27. Cyproheptadine	Periacthin®		—		0	0

in the mouse tail method is presented in Tables I–II. With this technique 24 out of 27 compounds were tested, 15 of which showed a phototoxic potency, all but one being phenothiazine derivatives. CPZ had the highest PI, followed by the other two chloro-phenothiazine derivatives (nos. 2 and 3). On the other hand, tricyclic compounds other than phenothiazine derivatives lacking nitrogen, sulphur, or both in their nucleus (Nos. 19–27) showed no activity.

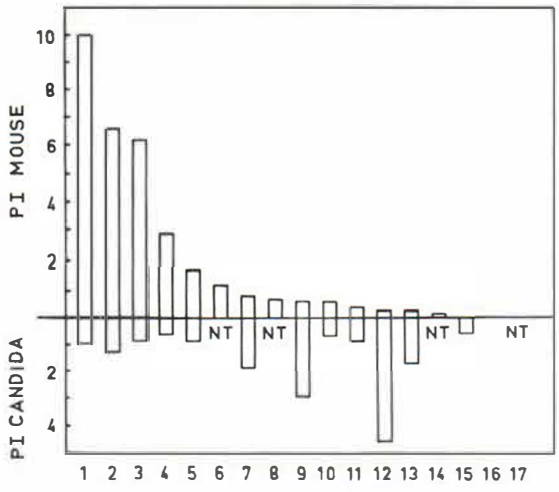


Fig. 1. Phototoxic index of phenothiazines as tested by the mouse tail and yeast methods. Nos. 1–17 refer to the substances in Table 1.

Importance of changes in molecular structure (in vivo)

The parent structure, phenothiazine itself (without any substituents), shows a definite though small phototoxic activity (Table 1: No. 14). However, the thioxanthenes (Nos. 19–21) lacking nitrogen, and the azepines (Nos. 22–24) lacking sulphur, are not phototoxic in the mouse. Similarly negative were the cycloheptenes (Nos. 25–27) having neither nitrogen nor sulphur in the ring system. It is interesting that when another nitrogen is introduced into the phenothiazine ring system the PI appears to increase (compare Nos. 9 and 18).

It was possible to evaluate the importance of substitution at 2-position (R_1) by comparing the results with various compounds in which the radicals at 10-position (R_2) were maintained (Table I). It is evident that the phototoxic activity was strongest with the chlorinated phenothiazines (Nos. 1–3) and that dechlorination results in a considerable reduction of this activity (No. 1 vs. No. 9). When CF_3 is substituted for chlorine in 2-position, PI diminishes (no. 2 vs. 7, and No. 3 vs. 5) and with $COCH_3$ there is no activity at all (No. 17). With other R_1-radicals too a certain phototoxicity is demonstrated (Nos. 4, 6, 8, 10).

Several compounds with substitution in 10-position (R_2) were also tested in the mouse. On the whole, changes in length or structure of the alkyl chain gave less PI variation than did corresponding substitutions at 2-position. Thus, the two chlorinated phenothiazines with a piperazine chain (nos. 2–3) showed a phototoxic activity only slightly less than CPZ. Correspondingly, small differences were seen between the non-substituted compounds (Nos. 9, 11, 12, 13, 14) with a side chain of varying length.

C. albicans technique (in vitro)

The in vitro technique with *Candida albicans* was also found to be a sensitive method for demonstrating drug phototoxicity. Thus, all phenothiazines phototoxic in the mouse and which could be tested in vitro were positive in this test, too (Fig. 1). On the other hand, some tricyclic compounds (Nos. 15, 19–21) were phototoxic in vitro only. Consequently, the yeast method appears to be useful as a screen test but the results should be checked with an in vivo method. This discrepancy in phototoxic action between in vivo and in vitro methods has been described earlier (8).

With the *Candida albicans* method, 21 out of 27 compounds were tested, 16 of which showed a phototoxic potency. Here, CPZ did not have the highest PI of the true phenothiazines, but approximately a median value. A very high PI was obtained for the thioxanthenes (Nos. 19–21) while the tricyclic compounds lacking sulphur in their ring systems were inactive.

Importance of changes in molecular structure (in vitro)

In contrast to findings with the mouse model, it was shown in vitro that the presence of both sulphur and nitrogen in the ring system was not a prerequisite for phototoxicity and that the presence of sulphur alone was quite sufficient. In fact, the addition of nitrogen to a sulphur-containing molecule reduced its phototoxic potency (No. 19 vs. 1, and 20 vs. 3). A similar relationship between molecular pattern and photodynamic action in vitro has earlier been described for the tricyclic compounds methylene blue, thiopyronin and pyronin (13). Introduction of a second nitrogen reduced the potency still further (No. 9 vs. 18), i.e. the reverse of the in vivo case.

With regard to the radical in 2-position of the various phenothiazines, it appears that chlorination or other substitution does not enhance the phototoxic action in vitro (No. 9 vs. 1). It was not possible to elucidate the importance of substitutions in 10-position.

Earlier comparisons of phenothiazine phototoxicity

Schulz et al. (11) studied the phototoxic activity of phenothiazines by using a variety of methods. After intraperitoneal injection in mice, CPZ was stronger than phenothiazine and mepazine. Epicutaneously in man, CPZ was found more potent than promethazine, mepazine, diethazine and 2-chlorophenothiazine. All compounds except the latter one were also active in an in vitro test using paramecia. Ison & Davis (3) found CPZ more phototoxic than prochlorperazine both in vivo (albino mice, hairless mice) and in vitro (yeast test). CPZ was found more potent than promethazine intracutaneously in man (5), and somewhat stronger than prochlorperazine epicutaneously in the guinea pig (12). These results are in agreement with our findings.

Photohemolysis has been reported for CPZ, prochlorperazine and alimemazine (4). Thioridazine was found to be phototoxic in tissue culture; surprisingly enough, this was not the case with CPZ (1).

Several phenothiazines have been reported to cause clinical phototoxicity (2, 9). For CPZ and thioridazine this side effect usually appears at daily doses of 400–600 mg (10). The phenothiazines with a high PI in our in vivo test are particularly well known to induce clinical reactions. Consequently, the mouse tail method may be recommended for preclinical testing of new phenothiazines.

CONCLUSIONS

A *sine qua non* for phototoxic action in vivo of the drug group studied appears to be an intact ring system including sulphur and nitrogen. Substitution in 2-position may enhance this capacity, and particularly so when the substituent is chlorine. Substitution in 10-position may also augment the phototoxic activity, though to a lesser degree. The results with the in vitro method correlated fairly well which those found in vivo, indicating that the yeast test might be used as a screening method prior to testing in vivo.

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