PHENOTHIAZINE PHOTOTOXICITY: TOXIC CHLORPROMAZINE PHOTOPRODUCTS

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The effect of preirradiated chlorpromazine (CPZ) solutions has been studied in vivo in the guinea pig and in vitro in a yeast culture. With intracutaneous administration of CPZ solutions preirradiated with long-wave ultraviolet radiation (UVA), an inflammatory response was attained equaling the response achieved when nonirradiated solutions were injected and the skin of the animals was subsequently irradiated with UVA.

Preirradiated CPZ was not toxic to the yeast culture. During irradiation the pH of the CPZ solution changed, indicating an accumulation of acid photoproducts. Nonphototoxic phenothiazines and related tricyclic drugs failed to change their pH following irradiation whereas phototoxic compounds behaved like CPZ. The low pH alone could not account for the inflammatory response in vivo, as neutralized irradiated CPZ solutions still induced inflammation, and acidified, nonirradiated solutions failed to cause a reaction.

CPZ phototoxicity seems mainly to be due to the effect of photoproducts. The nature of these is not known, but it is suggested that the stable free radical of CPZ might be an active principle.

Although phototoxic dermatitis is a well-documented side effect in patients treated with phenothiazines, little is known about the mechanism of chlorpromazine (CPZ) phototoxicity. It has not been established that any particular cell constituent is involved in the process, although CPZ binding to protein, microsomes [1], and nuclear DNA [2] has been demonstrated. Recently, in vitro studies have suggested that the phototoxic capacity could be attributed to the toxic effect of photoproducts of CPZ [3]. It was considered to be of interest to study in vivo the role of CPZ photoproducts in the elicitation of the inflammatory response, and also to investigate the nature of these photoproducts as manifested by a change in pH.

MATERIALS AND METHODS

In vivo. Female albino guinea pigs weighing around 300 gm were used as experimental animals.

Nonirradiated CPZ solutions. These were prepared using isotonic sodium chloride solution as solvent. Four concentrations of CPZ (Hibernal, AB Leo, Helsingborg, Sweden) were used: 0.05%, 0.01%, 0.002%, and 0.0004%. Pilot studies had shown that CPZ concentrations below 0.1% caused only negligible local inflammation. With a tuberculin syringe 0.1 cc of each solution was injected intracutaneously (ic) in the right flank of the animal that had been depilated with barium sulfide about 1 hr earlier. No skin irritation was evident at the time of injection. The wheal was then immediately irradiated with long-wave ultraviolet (UVA) from a 150-W xenon arc lamp (Osram XBO) equipped with Schott BG-38 and window-glass filters. The output of UVA measured with an optometer UDT-40X (United Detector Technology) was approximately 30 mW/cm², and the total dose delivered in 10 min at each test site was 18 J/cm².

Preirradiated CPZ solutions. The solutions earlier described were exposed to the same UVA dose from the same irradiation source while in glass tubes. Subsequently 0.1 cc of each of the exposed solutions was injected into corresponding sites on the right side of the same animals. In 4 animals, solutions prepared as above but, in addition, preirradiated with UVA from 3 blacklight tubes (Philips TL40W/08), total dose 100 J/cm², were injected ic also.

pH-adjusted CPZ solutions. Preirradiated CPZ solutions obtained as above were adjusted to neutrality by titration with a small volume of a 0.1 N solution of sodium hydroxide, not influencing the CPZ concentration noticeably. For the titration procedure and for the pH measurements, a pH meter PHM 26 C from Radiometer A/S, Copenhagen, Denmark was used. Nonirradiated solutions as above were adjusted to acidity (0.05% CPZ to pH 3.9, 0.01% CPZ to pH 4.7) using a 0.1 N solution of sodium hydrochloride. Of each solution, 0.1 cc was injected ic in the left flank of the 5 animals.

Control solutions. CPZ solutions with no UV exposure of either the solution or the animal were injected in the left side of the animal. The animals were kept away from daylight during 24 hr.

All tests were read after 24 hr using a scale where 0 = negative, 1 = just perceptible erythema, 2 = clear erythema, 3 = strong erythema and infiltration. Mean reactions of at least 5 animals were calculated. For statistical evaluation Student’s t-test was used throughout.

In vitro. In the in vitro study, a culture of Candida albicans was evenly spread over a Sabouraud-agar plate. Paper discs with a diameter of 0.8 cm were im-
mersed in a 2% solution of CPZ in ethanol/water and placed on the plate. One set of solutions had previously been irradiated with UVA (dose 54 J/cm²), one set was not irradiated. The plates with the unirradiated discs were then exposed to UVA from the 3 blacklight tubes earlier described. The exposure lasted for 18 hr giving a dose of about 65 J/cm². Control plates with preirradiated solution were kept in darkness. The zones of inhibited yeast growth around the discs were measured after 24 hr.

**pH measurements.** On irradiated solutions of CPZ and other phenothiazines and related tricyclic drugs, pH measurements were carried out with the pH-meter earlier described. CPZ was studied at the 4 concentrations used in the in vivo experiment, the other compounds as 0.1% solutions in isotonic sodium chloride only. Irradiation was performed with the xenon arc lamp fitted as above. pH measurements were made at intervals up to at least 20 min and in some instances also after a UVA dose of about 65 J/cm² from the blacklight tubes earlier described. Color changes were noted. The other phototoxic compounds tested were prochlorperazine, fluphenazine, and promethazine; the nonphototoxic compounds by in vivo assay were propiomazine, clomipramine, and chlorprotixene [4]. The chemical structure of the compounds is given in Table I.

**RESULTS**

**In vivo Experiment**

The lowest CPZ concentration giving a significant reaction together with UVA was 0.002% (Tab. II). Animals injected with this concentration and irradiated with UVA reacted slightly more strongly (p < 0.05) than animals injected with the preirradiated solution. By increasing the preirradiation dose given to 100 J/cm² this difference could be eliminated. At higher CPZ concentrations in vivo and in vitro irradiation caused equal reactions. Nonirradiated control injection sites showed no statistically significant reactions.

Nonirradiated CPZ solutions adjusted to the acid pH of the preirradiated solutions caused no inflammatory response when injected ic (Tab. III). On the other hand, preirradiated CPZ solutions adjusted to neutrality still evoked an inflammatory reaction. With CPZ 0.01%, however, this was somewhat less intense than with the acidic solutions (p < 0.01) as given in Table II.

**Table I. Chemical structure of the 7 phenothiazines and related compounds tested**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
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<tr>
<td>Chlorpromazine</td>
<td><img src="image1" alt="Structure" /></td>
<td>Cl</td>
<td></td>
<td>-CH₂CH₂CH₂N(CH₃)₂</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td><img src="image2" alt="Structure" /></td>
<td>Cl</td>
<td></td>
<td>-CH₂CH₂CH₂NCH₃</td>
</tr>
<tr>
<td>Fluphenazine</td>
<td><img src="image3" alt="Structure" /></td>
<td>CF₃</td>
<td></td>
<td>-CH₂CH₂CH₂NCH₂CH₂OH</td>
</tr>
<tr>
<td>Promethazine</td>
<td><img src="image4" alt="Structure" /></td>
<td></td>
<td></td>
<td>-CH₂CHN(CH₃)₂ CH₃</td>
</tr>
<tr>
<td>Propiomazine</td>
<td><img src="image5" alt="Structure" /></td>
<td>COCH₂CH₃</td>
<td></td>
<td>-CH₂CHN(CH₃)₂ CH₃</td>
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<tr>
<td>Chlorprotixene</td>
<td><img src="image6" alt="Structure" /></td>
<td>Cl</td>
<td></td>
<td>=CHCH₂CH₂N(CH₃)₂</td>
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<tr>
<td>Clomipramine</td>
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<td>Cl</td>
<td></td>
<td>-CH₂CH₂CH₂N(CH₃)₂</td>
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Table II. The effect of intracutaneously injected CPZ in the guinea pig

Solutions were irradiated before injection (in vitro) or after injection (in vivo) (n = 6). Mean reactions and standard deviation. For scale see text.

<table>
<thead>
<tr>
<th>CPZ %</th>
<th>No UVA</th>
<th>UVA in vivo 18 J</th>
<th>UVA in vitro</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>18 J</td>
</tr>
<tr>
<td>0.05</td>
<td>0.2 ± 0.4</td>
<td>3.0 ± 0</td>
<td>2.8 ± 0.4</td>
</tr>
<tr>
<td>0.01</td>
<td>0</td>
<td>2.5 ± 0.6</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>0.002</td>
<td>0</td>
<td>1.3 ± 0.8</td>
<td>0.2 ± 0.4</td>
</tr>
<tr>
<td>0.0004</td>
<td>0</td>
<td>0.2 ± 0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Table III. The effect of intracutaneously injected nonirradiated acidified and in vitro irradiated neutralized CPZ solutions in guinea pigs (n = 5)

<table>
<thead>
<tr>
<th>CPZ %</th>
<th>No UVA Acid pH</th>
<th>UVA exposure in vitro (dose 18 J) Neutral pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0.4 ± 0.9</td>
<td>3.0 ± 0</td>
</tr>
<tr>
<td>0.01</td>
<td>0.2 ± 0.5</td>
<td>0.8 ± 0</td>
</tr>
<tr>
<td>0.002</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.0004</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Mean reactions and standard deviation. For scale see text.

Fig. 2. pH of 6 different phenothiazines and related tricyclic drugs after irradiation with UVA. All solutions 0.1% in isotonic sodium chloride. Promethazine, prochlorperazine, and fluphenazine are phototoxic by in vivo assay; clomipramine, chlorprothixene, and propramzone are nonphototoxic. * = pH measured after an additional UVA dose of 65 J.

In Vitro Experiment

The in vitro experiment showed no toxic effect of preirradiated CPZ on the yeast cells in the dark control plates. The zones of inhibited yeast growth in the irradiated plates was 16 mm.

Measurements of pH

The measurements of pH in the CPZ solutions after irradiation showed a gradually increasing hydrogen ion concentration (Fig. 1). This was evident for the 0.05% and 0.01% solutions whereas the two lower concentrations showed no marked pH change after 20 min of irradiation. The nonphototoxic phenothiazines and related tricyclic drugs tested showed no decrease in pH even after an extended irradiation period, while the phototoxic compounds behaved like CPZ (Fig. 2). After the extended irradiation, CPZ and the other phototoxic phenothiazines all showed a marked color change to deep red-brown or violet. CPZ and promethazine also produced a precipitate. The color of the nonphototoxic substances did not change.

Discussion

The products formed from CPZ during UV irradiation are only partly known and the photochemical reaction is complex. In in vitro experiments, the solvent has been shown to influence to a high degree the type of photoproducts obtained [5]. In
water under aerobic conditions CPZ sulfoxide and promazine have been shown to be formed [6,7]. Also a free radical of CPZ with a long lifetime has been demonstrated by Forrest et al [8], an observation confirmed by others [9,10]. The CPZ free radical shows a remarkable stability which is pH dependent and increases with the hydrogen ion concentration [11,12]. Under anaerobic conditions promazine also appears [13], but polymerization processes dominate and the CPZ polymer has been claimed to be the major product. This compound caused no skin irritation when injected in humans [14].

In vitro CPZ photoproducts have been studied using red blood cells and mouse peritoneal macrophages [3]. Preirradiated CPZ was found to be toxic both to erythrocytes and to macrophages and the CPZ phototoxicity may result from the action of cytotoxic photoproducts on the plasma membrane. The lysosomes could not be shown to be involved in the process although evidence exists that CPZ has a high binding affinity and capacity for these cell constituents [1]. In the in vitro system, CPZ photoproducts were not toxic to the yeast cells. This could be due to an inability of the photoproducts to enter the cell through the cell wall. It is also possible that the agar medium might in some way interfere with the process.

In the guinea pig, on the other hand, CPZ photoproducts were clearly toxic and the intensity of the reaction was almost the same with in vitro-irradiated CPZ as when the irradiation took place in the skin. By increasing the in vitro irradiation the same degree of response as with in vivo irradiation could be attained (Tab. II). It is not possible to achieve identical conditions for the in vivo and the in vitro irradiation procedure and some factors differ, e.g., the exact UV dose reaching the energy-absorbing molecules after passing through epidermis and glass tube respectively, as well as the skin and room temperatures. It could also be expected that formation of the photoproducts close to their site of action would be favorable for a strong response. According to our results the effect of the photoproducts might account for most of the CPZ phototoxicity. In experiments using the mouse tail technique for demonstrating phototoxicity, intraperitoneally administered CPZ was shown to lose its phototoxic capacity when irradiated with UV prior to injection [7]. This would be expected if the effect was due to active photoproducts as the transport distance from peritoneum to tail skin is great. Probably under in vivo conditions the photoproducts have to be formed in close vicinity to the place of action.

During in vitro irradiation of CPZ solutions the pH was found to decrease (Fig. 1). The increasing hydrogen ion concentration can be explained on the basis of sulfoxidation [15], and also as a consequence of dechlorination producing hydrochloric acid [14]. At higher CPZ concentrations this pH change was rather rapid. To study whether this low pH alone could explain the test reactions, acidified nonirradiated and neutralized preirradiated CPZ solutions were injected in the guinea pig. The conclusion was that the change in hydrogen ion concentration makes no great contribution to the degree of reaction (Tab. III).

The formation of acid photoproducts, however, seems to be a prerequisite for phenothiazine phototoxicity, since phenothiazines and related tricyclic drugs known to be nonphototoxic in the mouse tail model [4] failed to show any pH or color change during irradiation. Phototoxic phenothiazines, on the other hand, showed the same photostability as CPZ (Fig. 2).

CPZ phototoxicity seems mainly to be due to the effect of photoproducts. Free radicals usually have a very short lifetime but in the case of CPZ and other phenothiazines they may be extremely long-lived. This biologically very active type of molecule might very well account for much of the inflammatory reaction in vivo, and because of its stability it could also explain the effect of in vitro irradiated solutions.

REFERENCES