Inhibition of Human Neutrophil Chemotaxis In Vitro by Phenothiazines and Related Compounds

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Chlorpromazine (CPZ) and three other phenothiazines and the structurally related antidepressant drugs imipramine and amitriptyline were found to depress human neutrophil chemotactic responsiveness. A 7 × 10⁻⁶ M solution of CPZ inhibited chemotaxis, whereas concentrations of the other tested drugs 10 to 1,000 times greater than this were needed to inhibit chemotaxis. This effect of CPZ could not, however, be demonstrated when testing neutrophils from patients treated with the drug. The inhibition of chemotaxis was reversible when CPZ-incubated neutrophils were washed before testing for chemotactic responsiveness. CPZ affects neutrophil function as well as other aspects of immune response.

Phenothiazine derivates are commonly used as tranquilizers; however, they show a wide variety of biological activity affecting the function of many organs in man (1). These compounds act in a reversible fashion on membranes of mammalian cells and inhibit a variety of cell surface phenomena (12). Chlorpromazine (CPZ) has been shown to inhibit several lymphocyte responses, including the proliferative response and generation of cytotoxic lymphocytes in mixed lymphocyte cultures (2). Rutu in 1972 showed that a number of phenothiazines inhibited neutrophil phagocytosis (9). Diminished resistance to infection has been reported in patients on high doses of CPZ (7, 13).

Since chemotactic responsiveness is an important function of neutrophils in host resistance, the effect of phenothiazines and the structurally closely related antidepressant drugs imipramine and amitriptyline on neutrophil chemotaxis was studied.

MATERIALS AND METHODS

Leukocytes from healthy young adults and from four patients treated with phenothiazine drugs were separated by allowing heparinized venous blood to sediment spontaneously for 45 min in plastic syringes. The leukocytes were separated into plastic tubes (17 by 100 mm) (Falcon, Oxnard, Calif.). The leukocytes were separated from platelets by centrifugation at 400 × g for 5 min and were suspended in Hanks balanced salt solution (Hanks BSS) at a concentration of 4 × 10⁶ to 8 × 10⁶ polymorphonuclear leukocytes per ml. Leukocytes from one donor were used for all assays done on the same day.

The following phenothiazine derivatives were studied: CPZ and prochlorperazine (Smith, Klein and French Laboratories, Philadelphia, Pa.), perphenazine (Schering Corp., Kenilworth, N.J.), amitriptyline hydrochloride (Merck Sharp & Dohme, West Point, Pa.), thioridazine (Sandoz, Inc., East Hanover, N.J.), and imipramine (Ciba-Geigy, Summit, N.Y.). The drugs were diluted in Hanks BSS with 0.3 mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.3.

Experimental procedures. Equal volumes of leukocyte suspension and drug solutions at various concentrations or Hanks BSS alone were incubated for 30 min at 37°C. After incubation, a volume of 0.4 ml of leukocyte suspension was placed in the upper compartment of Boyden chambers on the surface of 5-µg cellulose acetate filter (Millipore Corp.). The lower compartment was filled with chemotactic attractant, a culture supernatant from an overnight culture of Escherichia coli (4). The chambers were incubated for 60 min at 37°C, and the filters were fixed and stained as previously described (4). The distance of migration of neutrophils from the top of the filter was measured by determining the leading front of neutrophils in the filter using a magnification of ×300 (4). The effect of drugs on chemotaxis is expressed as a percentage of the distance of migration of neutrophils preincubated with drugs compared with distance of migration by neutrophils not incubated with drugs.

RESULTS

There was inhibition of neutrophil chemotaxis by all of the compounds tested, although there was a marked difference in the concentrations necessary for this effect (Fig. 1). CPZ was markedly inhibitory (63%) at a concentration of 5 µg/ml (1.4 × 10⁻⁶ M). The concentration of other phenothiazines necessary to inhibit neu-
DISCUSSION

Ruutu reported that CPZ and related compounds depressed leukocyte phagocytosis and bacterial killing, and he considered this to be an effect of CPZ on the cell membranes, since washing the leukocytes completely reversed the inhibition (9, 10). Furthermore, the drug did not interfere with opsonization (9, 10). The concentrations of CPZ that depressed phagocytosis were higher than the concentrations that depressed chemotaxis. At a concentration of $2 \times 10^{-5}$ M, phagocytosis was 90% of the normal; with $5 \times 10^{-5}$ M (17.8 $\mu$g/ml), phagocytosis was 80% of the normal (9).

Recently Ferguson et al. (2) reported that CPZ inhibits allogenic stimulation of the proliferative response in mixed lymphocyte culture and inhibits the mixed lymphocyte culture generation of cytotoxic lymphocytes. At a CPZ concentration of 1.8 $\mu$g/ml, there was a 50% inhibition of $[^3]$H]thymidine uptake in mixed lymphocyte culture.

Chemotaxis was inhibited by concentrations as low as 2.5 $\mu$g/ml and thus appears to be more sensitive to the effect of CPZ than phagocytosis. Indeed, Ruutu and Collan demonstrated that CPZ at $2 \times 10^{-5}$ M inhibited pseudopod formation by the leukocytes, although phagocytosis remained almost normal (11). This difference in concentration of CPZ necessary to effect chemotaxis and phagocytosis might be a useful laboratory tool to study neutrophil phagocytosis under conditions where there is little chemotaxis.

The chemotaxis-depressing effect varied between different phenothiazine compounds; prochlorperazine was only slightly inhibitory at 25 $\mu$g/ml. Amitriptyline and imipramine structurally resemble the phenothiazines and are used in similar clinical conditions; however, these drugs were inhibitory in higher concentrations than CPZ. Ten micrograms of amitriptyline

<table>
<thead>
<tr>
<th>Patient</th>
<th>Daily dose of CPZ or THI</th>
<th>Chemotaxis ($\mu$m)</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CPZ (2,400 mg)</td>
<td>90</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>CPZ (800 mg)</td>
<td>103</td>
<td>88</td>
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</tr>
<tr>
<td>3</td>
<td>CPZ (1,200 mg)</td>
<td>89</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>THI (300 mg)</td>
<td>79</td>
<td>100</td>
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<tr>
<td>Control</td>
<td></td>
<td>93</td>
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<tr>
<td>Control</td>
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<td>92</td>
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* Expressed as the distance traveled by the leading front.

Fig. 1. Effect of phenothiazines on leukocyte chemotactic responsiveness. The effect is expressed as a percentage (mean and range) of the chemotaxis of leukocytes not incubated with any drugs. 1 = chlorpromazine; 2 = prochlorperazine; 3 = perphenazine; 4 = thioridazine; 5 = amitriptyline; 6 = imipramine.

trophil chemotaxis was $25 \mu$g/ml ($6 \times 10^{-3}$ to $8 \times 10^{-3}$ M) or higher, and with imipramine and amitriptyline a concentration of $50 \mu$g/ml ($1.5 \times 10^{-4}$ M) or higher was required for an inhibitory effect. No gross morphological changes were observed in incubated cells examined by light microscope, and more than 99% of the neutrophils incubated with $12.5 \mu$g of CPZ per ml excluded trypan blue.

Inhibition of chemotaxis by phenothiazines was reversible since normal chemotaxis was observed when leukocytes that had been incubated for 30 min with $12.5 \mu$g of CPZ per ml ($3.5 \times 10^{-4}$ M) were washed in Hanks BSS and resuspended in plasma.

To investigate a possible correlation between these in vitro results and an in vivo effect of the compounds, we evaluated the chemotaxis of leukocytes from four adults, two men and two women, who were being treated with phenothiazine derivatives. The patients were receiving $400 \text{mg}$ of CPZ twice, $300 \text{mg}$ three times, and $600 \text{mg}$ four times daily or $100 \text{mg}$ of thioridazine three times daily, respectively. Blood was drawn in the morning before the drugs were given and again 90 to 120 min after the first dose of the drugs. As shown in Table 1, chemotaxis of their leukocytes was not depressed by chlorpromazine or thioridazine at therapeutic dosages.

Table 1. Chemotaxis of leukocytes from four patients before and 90 to 120 min after oral medication with chlorpromazine (CPZ) or thioridazine (THI)

<table>
<thead>
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<th>Patient</th>
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* Expressed as the distance traveled by the leading front.
per milliliter \((3.2 \times 10^{-3} \text{ M})\) did not depress chemotaxis, and 50 \(\mu g\) of imipramine per ml \((1.6 \times 10^{-4} \text{ M})\) only slightly depressed chemotaxis (Fig. 1).

The concentration of CPZ necessary to demonstrate inhibition of chemotaxis was 2 to 10 times higher than peak plasma levels found in patients receiving the drugs (5, 6). However, the drug is rapidly metabolized, and serum concentrations of the drug and pharmacologically active metabolites of it range from 4 to 22 \(\mu g/ml\) in patients receiving the drug orally (6). Measurement of serum concentrations of CPZ are difficult and may not correspond with clinical effectiveness of the drug in patients on long-term treatment (8). CPZ is accumulated in certain tissues, particularly the lungs, where concentrations of up to 80 \(\mu g/g\) of wet tissue are found (3). The in vitro effect of CPZ on neutrophil function may well have a clinical counterpart in vivo and may bear relevance to the suggested association between pulmonary infection and CPZ treatment (7, 13).

**LITERATURE CITED**


3. Forrest, I. S., A. G. Bolt, and M. T. Serra. 1968. Distribution of chlorpromazine metabolites in selected or-


