Depressant effects of major tranquillizers on contact hypersensitivity to picryl chloride in the mouse

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Summary. Chlorpromazine, levomepromazine, thioproperazine, promethazine and haloperidol were found to significantly depress contact hypersensitivity to picryl chloride in the mouse, a model of cell-mediated immune response.

Among untoward reactions to major tranquillizers, bacterial infections, in particular those affecting the bronchopulmonary tract, have been repeatedly reported[2-5]. Chlorpromazine and related compounds are known to alter phagocytosis and bacterial killing both in vivo and in vitro[6-9], to decrease primary and secondary antibody response[7,10,11] and finally, to lower animal resistance to experimental infections[12,13].

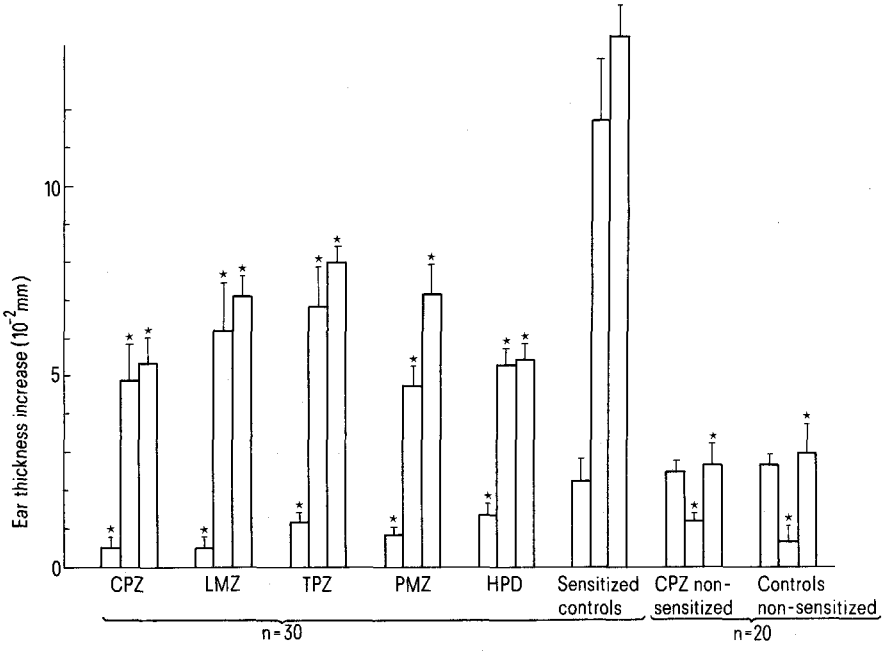
By contrast, very few data are currently available concerning the effects of such components on cell-mediated immunity. We have therefore studied the properties of the more widely used phenothiazines: chlorpromazine (CPZ), levomepromazine (LMZ) and thioproperazine (TPZ) on contact hypersensitivity to picryl chloride in the mouse, a valuable model of in vivo cell-mediated immunity in that species[14]. Their effects were compared with those of promethazine (PMZ), a well-documented immunosuppressant phenothiazine[15,16] used as a reference, and with those of haloperidol (HPD), a butyrophenone-derivative whose immunopharmacological properties are little known.

Methods. Outbred 3-month-old Swiss mice of both sexes were randomly used throughout the study. Sensitization, challenge and quantification of contact hypersensitivity were performed as previously described by Asherson and Ptak[17]: mice were sensitized epicutaneously with 0.1 ml of 8% picryl chloride in acetone applied to the clipped abdomen. Epicutaneous challenge was performed 7 days later with 1% picryl chloride dissolved in olive oil. 0.05 ml were applied to both sides of the ear and increased ear thickness was measured with an engineer's micrometer 4, 24 and 48 h after challenge. 2 measurements were made on each occasion by 2 different observers and the mean of these 2 measurements was calculated. The results were expressed as the increase in thickness of the ear measured in units of 10^{-2} mm. Groups of 30 mice each were treated, the drugs being given daily from 3 days before sensitization until 48 h after challenge. All drugs were dissolved in saline and administered i.v. through the tail vein once a day. The daily doses were 2 mg/kg for CPZ and LMZ, 0.5 mg/kg for TPZ, 1.5 mg/kg for PMZ and 0.3 mg/kg for HPD, taking into account the relative clinical effectiveness of these drugs[18]. An additional group of 30 mice received 0.5 ml i.v. saline daily as controls. Finally, 20 nonsensitized mice were challenged with 1% picryl chloride and their ear thickness increase measured at 4, 24 and 48 h while on CPZ treatment, and 20 nonsensitized nontreated mice were challenged similarly. Student's t-test was used in the statistical comparison of the different groups.

Results. The mean increases of ear thickness ± SEM in treated and control mice are given in the figure. All 5 drugs developed marked depressant effects on contact hypersensitivity to picryl chloride as compared to sensitized controls ($p < 0.001$) whenever measurements of ear thickness were performed (at 4, 24 and 48 h). However, CPZ and HPD were found to exert the most potent effect while TPZ was the least active.

Discussion. These results suggest that major tranquillizers do exert a depressive effect on cell-mediated immunity. Interestingly, PMZ was not more potent in this respect than other phenothiazine derivatives as might have been expected from previously published reports[15,16]. However, our results are somewhat in disagreement with those of Boranic et al.[19] who did not find any modification of antibody production in haloperidol-treated mice.

Our schedule of drug administration does not provide any clue as to the possible mechanism of this immunodepressive action of major tranquillizers. Previous data suggest



Effect of chlorpromazine (CPZ), levomepromazine (LMZ), thioproperazine (TPZ), promethazine (PMZ) and haloperidol (HPD) on contact hypersensitivity to picryl chloride as measured 4 h (left column), 24 h (middle column) and 48 h (right column) after challenge. Significance at the $p < 0.001$ level by comparison to sensitized controls is expressed as *.

that most CPZ effects are related to cell-surface alterations[20], thus providing an explanation for the observed inhibition of concanavalin A-induced lymphocyte blastogenesis[21] and generation of cytotoxic lymphocytes in mixed lymphocyte culture[22] by CPZ. This latter drug and haloperidol were postulated to decrease DNA synthesis in cultured lymphocytes by the same mechanism[23]. Furthermore, the CPZ-induced decrease of calcium movements[24] may explain the observed depression of ear thickness at 4 h since previous workers have demonstrated the involvement of mast cell degranulation in the early events of contact hypersensitivity to picryl chloride in the mouse[24]. A decrease of the nonspecific irritative properties of picryl chloride is probably not involved, as suggested by the comparison of nonsensitized, nontreated and CPZ-treated mice. While antihistaminic properties deserve attention with respect to delayed hypersensitivity reactions[25] depressant effects of HPD throw more emphasis on the role of stress[26].

Our data suggest that major tranquillizers are able to depress in vivo cell-mediated immunity, the consequences of which deserve further investigation with respect to psychiatric diseases and their pharmacological management.

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Enzyme-linked immunosorbent assay to detect anti-sea nettle venom antibodies

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Summary. A micro enzyme-linked immunosorbent assay has been developed to detect serum antibodies to sea nettle venom. This sensitive assay is useful for monitoring specific immunological response in envenomated patients or in immunized animals.

The sea nettle *(Chrysaora quinquecirrha)* injects a nematocyst venom into the skin of bathers who contact its long tentacles. The sting of this coelenterate results in painful cutaneous lesions, local muscle cramps and corneal ulcerations[3]. Both repeatedly stung individuals and challenged animals possessed elevated levels of anti-sea nettle venom serum antibodies[4,5].

During the last decade, investigations in this laboratory on the sea nettle have revealed the presence of degradative enzymes and pharmacologically active components within its nematocyst venom[3]. In this paper the details of a sensitive enzyme-linked immunosorbent assay (ELISA) are reported.

Materials and methods. All inorganic and organic chemicals were reagent grade quality or better. Alkaline phosphatase (type VII), p-nitrophenylphosphate and bovine serum albumin (BSA, RIA grade) were obtained from Sigma Chemical Co. (St. Louis, MO). Goat anti-human IgG and IgM were products of Miles Laboratories, Inc. (Elkhart, IN). Goat anti-guinea-pig IgG (heavy and light chains) were obtained from Cappel Laboratories, Inc. (Cochranville, PA). Guinea-pigs were supplied by Rockland Farms (Gilbertville, PA).

Preparation of the antigen. Tentacles were removed from live *C. quinquecirpha* and nematocyst venom (SU) (25 mg protein/ml; 1 mouse i.v. $LD_{50}=0.06$ ml) was prepared as previously described[6,7]. For immunization of guinea-pigs, the crude venom, free of other tissue components, was coupled with starch as follows: 10 g of soluble starch (in 10 ml of 2 M sodium carbonate) and 4 g of cyanogen bromide (in 4 ml of acetonitrile) were allowed to react for 3 min in an ice bath. The reaction was stopped with 20 ml of cold coupling buffer (0.1 M sodium bicarbonate, 1 M sodium chloride, pH 8.5). The mixture was then shaken for 5 min and the slurry filtered on an Amicon filter (XM 50) to remove buffer and solvents. The activated starch 'cake' was resuspended in an equal volume of coupling buffer and 0.6 ml of SU was added. After stirring overnight (16 h) at