A drug-specific leuco-agglutinin in a fatal case of agranulocytosis due to chlorpromazine

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SYNOPSIS A fatal case of agranulocytosis due to chlorpromazine is reported. Mechanisms other than immunological are generally believed to be responsible for chlorpromazine-induced agranulocytosis. However, the demonstration of a drug-specific leuco-agglutinin in the serum of this patient suggests that an immunological mechanism was responsible for the agranulocytosis.

The occurrence of agranulocytosis as a complication of chlorpromazine (Thorazine®) therapy has been well established but its mechanism remains obscure. Most instances have been observed in psychiatric patients receiving large cumulative doses of chlorpromazine. There is, however, considerable variation both in the duration of therapy and in the dose of the drug administered before agranulocytosis develops. This report concerns a fatal case of agranulocytosis due to chlorpromazine in which a drug-specific leuco-agglutinin was demonstrated.

CASE HISTORY

A 59-year-old man had been treated for asthma in another hospital from 12 May 1959 and had received chlorpromazine from 4 June to 30 July. During this period he received a total of 14 g. of chlorpromazine, the highest daily dose being 250 mg. Additional medication consisted of Tedral,³ decamethasone, digitalis, secobarbital sodium, and Caytine.⁴ A blood count on 17 June showed a haemoglobin of 12.8 g./100 ml. and a white blood cell count of 10,000/c.mm. (52% segmented neutrophils, 3% band cells, 12% eosinophils, 24% lymphocytes, and 9% monocytes).

When admitted to the Cleveland Clinic Hospital on 1 August 1959 because of sore throat, fever, and weakness of two days' duration, he was acutely ill and disorientated. Rectal temperature was 103.4 F., pulse rate 116, and blood pressure 98/56 mm. of Hg. The patient had a large furuncle on the neck and severe pharyngitis. The heart, lungs, and abdomen were normal but there

was mild nuchal rigidity, left homonymous hemianopsia, right papilloedema, right forced grasp and extensor plantar response, left central facial weakness, and left hemiparesis.

Laboratory studies showed a haemoglobin of 10.7 g./100 ml. and an initial white blood cell count of 200/c.mm., all of which were lymphocytes. Subsequent white blood cell counts of 400/c.mm., consisting entirely of lymphocytes, were obtained on 4, 5, and 6 August. The platelet count was 300,000/c.mm. A urine analysis was normal and a urine culture was sterile. The blood urea was 65 mg./100 ml. The serum total protein was 5.4 g. with 2.5 g. of albumin and 2.9 g. of globulin/100 ml. Results of a test for lupus erythematosus and of Wassermann and Kahn tests were negative. Blood cultures were positive for *Escherichia coli*. A sternal marrow aspirate was cellular showing no granulocytic precursors; other marrow elements were normal. X-ray examination showed the chest and the skull to be normal. An electrocardiogram showed myocardial changes. Two cerebrospinal fluid examinations revealed no abnormality.

Treatment consisted of 400,000 units of penicillin every six hours, 0.5 g. of streptomycin every 12 hours, 50 mg. of cortisone every 12 hours, and later 1 g. of chloramphenicol every eight hours. The patient continued to be febrile, had several generalized convulsions, became comatose, and died on 7 August 1959.

The clinical diagnosis was agranulocytosis secondary to chlorpromazine with *E. coli* septicaemia. Necropsy examination revealed severe generalized arteriosclerosis, two healed myocardial infarcts, and areas of ischaemic necrosis in the right occipital lobe of the brain, the largest being 0.5 cm. in diameter.

LEUCO-AGGLUTININ STUDIES

Leuco-agglutinin studies were performed on the patient's serum on the seventh and eighth days after discontinuing chlorpromazine therapy. The patient's serum was tested for leuco-agglutinins by a modification of the method

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described by Walford, Petersen, and Doyle (1957). In essence this test consists in mixing the patient's serum with normal leucocytes and noting whether or not agglutination occurs. Blood was drawn into a sterile non-siliconized glass test tube and was allowed to clot at 37°C. The serum obtained by centrifugation was inactivated at 56°C. for 30 minutes, and 1 in 4 and 1 in 16 saline dilutions were made. White blood cell suspensions were obtained from donors having the same ABO blood group as the sera being tested. Sedimentation of the red blood cells from approximately 15 ml. of defibrinated blood was accomplished by the addition of 1 part dextran to 3 parts blood. This mixture was allowed to stand at room temperature until the supernatant contained a few red blood cells. The supernatant was transferred to a non-siliconized glass test tube and its white blood cell count adjusted to approximately 10,000/c.mm. by the removal or addition of serum. The test was performed promptly after separating the leucocytes. A series of 10 by 75 mm. glass test tubes was set up containing 0.1 ml. of serum, 0.1 ml of saline solution or chlorpromazine in saline solution, and 0.1 ml. of the leucocyte suspension (Table I). The tubes were shaken and were placed in a 37°C. water bath for one hour. Each tube was then vigorously agitated by tapping several times against a hard surface to break up any non-specific agglutinates. A drop of the contents was examined for leucocyte agglutination, under a cover-slip, at low-power magnification. The degree of agglutination was graded 0 to 4, 0 indicating no leucocyte agglutination, 1 indicating the presence of numerous small clumps, and 4 indicating that all the leucocytes had clumped into a few large aggregates.

Leucocyte agglutination occurred only in those tubes containing both the patient's serum and chlorpromazine and was slightly stronger when the concentration of chlorpromazine was 1.0 mg./100 ml. (Table I). A further 14 random sera were tested in various combinations with six white blood cell suspensions. No agglutination was seen with or without the addition of a 1 mg./100 ml. solution of chlorpromazine. No serum from a patient with *E. coli* bacteraemia was available for testing but leuco-agglutinins were not demonstrated in the serum

of a patient with *Aerobacter aerogenes* septicaemia with or without the addition of chlorpromazine.

DISCUSSION

The mechanism of chlorpromazine-induced agranulocytosis is not understood. Pisciotta, Ebbe, Lennon, Metzger, and Madison (1958) found no evidence to support an immunological basis for this disorder. Similar absence of leucocyte antibodies has been reported by other authors (Korst, 1959; Hollister, 1958; Rotstein, Frick, and Schiele, 1955). The fact that agranulocytosis does not recur when small amounts of the drug are given again but may do so with large doses has been cited as further evidence of the non-immune nature of this reaction. Pisciotta *et al.* (1958) considered that individual biochemical or enzymatic idiosyncrasies played a major role. However, various studies *in vitro* and *in vivo* of the leucocytes from patients with agranulocytosis due to chlorpromazine have failed to demonstrate a block in the influx of sulphur-35 labelled L-cystine and L-methionine into the leucocyte (Pisciotta, Ebbe, Daly, Ruwaldt, Glaser, and Metzger, 1960), and their glutathione content has been found to be normal (Pisciotta and Daly, 1960). Recently Pisciotta and Keldahl (1962) showed that excessive concentrations of chlorpromazine partially inhibited the influx of thymidine and uridine into granulocytes of all patients in whom agranulocytosis developed due to this drug, although not in patients receiving chlorpromazine who showed no alteration in their leucocyte counts.

The fact that leuco-agglutinins have been demonstrated in so few instances of agranulocytosis due to chlorpromazine (Cunningham and Hannah (1960) cite Meyler (1958) as reporting leuco-agglutinins in the serum of one patient) suggests that an immuno-

TABLE I
RESULTS OF LEUCO-AGGLUTININ TESTS

Serum (0.1 ml.)		Substance Added (0.1 ml.)		Leucocyte Agglutination, Grades 0 to 4	
Source	Dilution	Saline Solution (0.8%)	Chlorpromazine Solution		Date of Test
			(1.0 mg./100 ml.)	(0.5 mg./100 ml.)	
					6 Aug. 7 Aug.
Normal	Undiluted 1:4, 1:16	+	—	—	0 0
Normal	Undiluted, 1:4, 1:16	—	+	—	0 0
Normal	Undiluted, 1:4, 1:16	—	—	+	0 0
Patient	Undiluted, 1:4, 1:16	+	—	—	0 0
Patient	Undiluted	—	+	—	4 4
Patient	1:4	—	+	—	2 3
Patient	1:16	—	+	—	2 2
Patient	Undiluted	—	—	+	2 3
Patient	1:4	—	—	+	1 1
Patient	1:16	—	—	+	1 1

logical mechanism is only responsible on rare occasions.

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