In Vitro Effects of Chlorpromazine and Meprobamate on Blast Transformation and Chromosomes (35229)

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Chromosome abnormalities have been reported in human lymphocytes exposed to psychotropic drugs such as LSD-25 (1, 2), thalidomide (3) and caffeine (4, 5). Chlorpromazine (CPZ) and meprobamate are now widely used not only in psychiatric, but in general medical practice. The following experiments were performed to ascertain whether or not CPZ and meprobamate are able to produce chromosome aberrations *in vitro*, and to observe the effects of the drugs on blast transformation and mitosis.

Materials and Methods. For the present experiment leukocytes were obtained from the venous blood of 10 normal adults, ranging in age from 26 to 34 years. Each culture flask contained 7.9 ml of culture medium (Eagle's MEM), 2 ml of autologous serum, 0.1 ml of phytohemagglutinin (PHA, Welcome Research Laboratory), and 12–15 $\times$ 10^6 leukocytes. Chlorpromazine hydrochloride was dissolved in water to provide final concentrations of 10^{-4}, 5×10^{-5}, 2×10^{-5}, 10^{-5}, and 2×10^{-6} *M*. Final concentrations of 10^{-3}, 5×10^{-4} and 10^{-4} *M* of meprobamate were prepared by dissolving it in culture medium. The drugs were added at the start of culture and at 4, 18, 30, and 48 hr. The control flasks were treated with a comparable volume of saline. The leukocytes were cultured at 36.5° for 72 hr. Two hr before the harvest, 2 ml of the medium were aspirated from each flask and sedimented, and smears were made of the sedimented cells. 0.2 μ/ml of colchicine were then added to the remaining culture. After 2 hr, the leukocytes were washed in 5 ml of Eagle's balanced salt solution and were then suspended in 5 ml of 0.95% sodium citrate. The hypotonic cell suspension was placed in an incubator (36.5°) for 30 min. The cells were then sedimented and resuspended in 5 ml of freshly prepared 1:1 ethanol:glacial acetic acid fixative for 10 min. The fixed cells were dispersed on slides, air dried, and stained with Giemsa's for 10 min. PHA-stimulated cells were differentiated from unstimulated cells by their large size, abundant basophilic cytoplasm, diffuse nuclear chromatin, and one or more nucleoli (6, 7).

Results. Table I shows the amount of chromosome aberrations in human leukocytes treated with either various concentrations of CPZ or treated with 2×10^{-5} *M* of CPZ at different times. Five hundred metaphases were observed in the control slides and approximately 4000 metaphases were scored in the CPZ-treated group. In the controls, 0.8% of cells exhibited chromosome abnormalities, which were all single chromatid breaks. In the cultures treated with various concentrations of CPZ the percentage of the abnormalities ranged between 0.66 and 1.4. The abnormalities scored in the CPZ-treated group were mainly chromatid breaks and minute fragments and very few iso-chromatid breaks. No chromatid or chromosome type of interchange were observed. No significant difference was found in the percentage of abnormalities between controls and the CPZ-treated group ($p<0.1$).

An inhibitory effect on blast transformation in 72-hr cultures of concentrations of CPZ varying from 10^{-4} to 10^{-5} *M* was found (Fig. 1). CPZ in concentration over 10^{-4} *M* decreased the total cell numbers in the flask and produced many ghost cells on smears. While 2×10^{-6} *M* of CPZ had no measura-

TABLE I. Chromosome Aberrations of Human Leukocytes Treated with Various Concentrations of CPZ and Treated with 2×10^{-5} *M* CPZ added at Various Culture Times

	Conc (*M*) of CPZ				Time of addition of 2×10^{-5} *M* CPZ (hr):				
	Nontreated	5×10^{-5}	2×10^{-5}	10^{-5}	2×10^{-6}	0	4	18	30	48
Total cells with abnormal chromosomes	4/500	1/136	6/500	7/500	2/300	6/500	6/500	5/500	3/500	5/500
Abnormal cells (%)	0.8	0.73	1.2	1.4	0.66	1.2	1.2	1.0	0.6	1.0

ble effect, 5×10^{-5}, 2×10^{-5}, and 10^{-5} *M* of CPZ significantly diminished blast transformation in 72-hr cultures. Differences in the number of blasts between control and CPZ-treated cultures were highly significant.

Figure 2 shows the percentage of PHA-stimulated cells in leukocytes cultures treated with CPZ (2×10^{-5} *M*) at various times of culture. It was found that the transformation of lymphocytes to blast cells was suppressed when CPZ was added into the medium within the first 18 hr of the culture ($p<0.05$). Percentage transformation was not significantly different from controls when the drug was added 30 to 48 hr after the start of the culture.

The mitotic index was measured after various combinations of exposure to CPZ and colchicine (Figs. 3 and 4). Figure 3 shows the mitotic index of the cultures treated with various concentrations of CPZ for 72 hr and without terminal addition of colchicine. Treatment with 2×10^{-5} *M* CPZ markedly increased the mitotic index ($p<0.01$). There was no significant difference between the control culture and cultures treated with 2×10^{-6} *M* CPZ. 10^{-5} *M* CPZ gave a small but significant increase ($p<0.02$). Mitotic indices were increased when cultures were treated with 2×10^{-5} *M* CPZ or colchicine for the last 2 hr compared with control flasks containing PHA only for 72 hr (Fig. 4). Both differences were significant ($p<0.02$ and $p<0.01$), but colchicine gave a higher mitotic index 6.56 ± 1.72% compared with 4.09 ± 1.26% for CPZ and 1.82 ± 0.53 for PHA only. Treatment with CPZ (2×10^{-5}) or colchicine for 72 hr gave values of 9.51 ± 2.23% and 19.8 ± 2.72%, respectively. When colchicine was added 2 hr before the harvest to a culture exposed to CPZ for the entire 72 hr period, a value of 12.73 ± 3.61% was obtained.

The cultures treated with CPZ (2×10^{-5} and 10^{-5} *M*) for 3 days showed a high degree of C-effect, *i.e.*, mitotic spindle suppression of the metaphases and anaphases, and highly contracted chromosomes.

The results of the experiments with meprobamate are given in Table II. Five hundred metaphases in the control and 2500 in the meprobamate-treated group were exam-

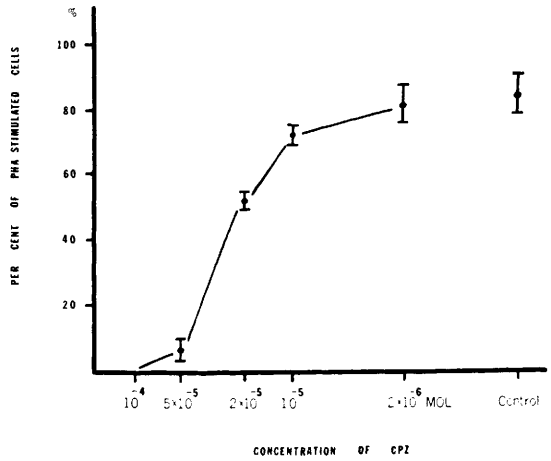

FIG. 1. Suppression of blast transformation at various concentrations of CPZ in PHA-stimulated cultures at 72 hr: vertical lines indicate ±1 SD.

ined. In both groups, most of the aberrations observed were chromatid breaks and small acentric fragments. The frequencies of abnormal chromosomes were within 1% in both groups. No significant difference was found in the percentage of abnormalities between control and meprobamate-treated groups ($p<0.1$). Meprobamate, in final concentrations from 10^{-3} to 10^{-4} *M*, did not influence the rate of blast transformation and mitotic index, as is the case with the CPZ-treated cultures.

Discussion. The results indicate that chlorpromazine and meprobamate do not raise the rate of chromosomal aberrations in primary cultures of normal human leukocytes, when added to the culture medium at various times and in various concentrations.

Cohen *et al.* (1) reported 4–17.5% of chromosome aberrations from lymphocytes in some patients who had been taking moderate quantities of CPZ (200 mg/day) for over 3 weeks. Plasma levels of CPA after single administration of the drug in chronic users of CPA were measured by Curry *et al.* (8). They showed that the maximum plasma level of CPZ was reached 3 hr after oral administration in most patients and that CPZ was rapidly eliminated from the plasma. The longest half-life for CPZ in their experiments was 6 hr. There was considerable variation in patients on similar dosage regimens. The

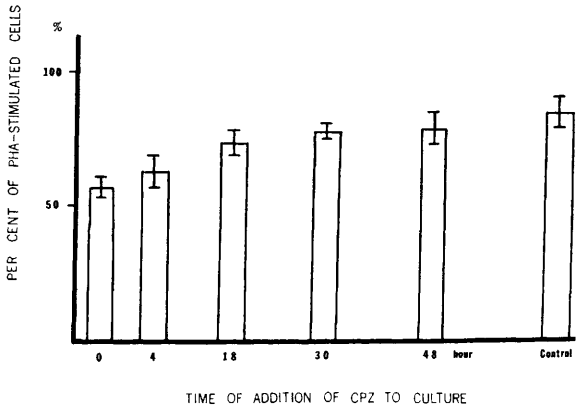

FIG. 2. Effect of delayed addition of CPZ (2×10^{-5} *M*) to PHA-stimulated culture: Blast transformation was suppressed when CPZ was added to the medium within the first 18 hr of culture.

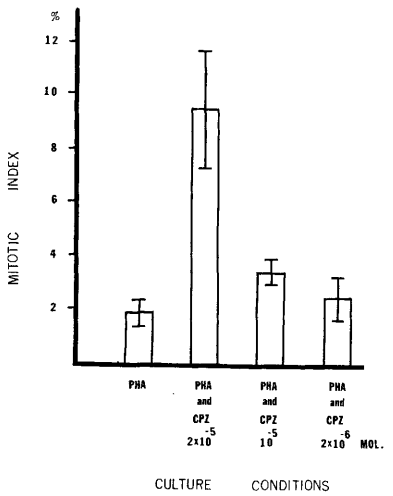

FIG. 3. Dose effect of CPZ on mitotic index of PHA-stimulated cultures at 72 hr: cultures harvested without pretreatment with colchicine.

plasma levels at 3 hr after administration ranged between 45–105 ng/ml in patients given 2.1–2.7 mg/kg of CPZ and 68–248 ng/ml in patients on 5.9–10.9 mg/kg. 248 ng/ml of CPZ equals approximately 10^{-6} *M*. The CPZ concentration, 10^{-6} *M*, used in the present study thus appears to be very close to the plasma concentrations obtained after pharmacological use of the drug. In the present *in vitro* study 2×10^{-6} *M* of CPZ did not raise the rate of chromosome abnormalities. The possibility exists however, that CPZ may be metabolized *in vivo* to some other form which may cause chromosome aberrations. Therefore, further investigation of chromosome aberrations of peripheral leukocytes from chronic users of CPZ may be desirable.

In the meprobamate experiment no chromosomal aberrations and no effects on blast transformation and mitotic index were found in the cultures treated with 10^{-3} of meprobamate (Table II). The peak levels in the serum after the administration of the normal dose (400–800 mg) of meprobamate are 0.8 to 1.6 mg/100 ml, less than 10^{-4} *M* (9). At 10 times this level of meprobamate no *in vitro* effect was seen.

The present findings indicate that CPZ decreases the number of PHA-stimulated cells in 72-hr cultures. The inhibitory effect was closely related to the concentration of CPZ in the medium and to the time of addition of the drug. These results confirm in part the data of Pisciotta *et al.* (10), in that blast transformation was inhibited in both studies. The present data also indicate that CPZ must be added early to reduce blast transformation from the control value of 85% to about 56%. If added after 18 hr the effect is minimal. In contrast to earlier findings, we found the mitotic index increased, apparently due to metaphase arrest caused by mitotic spindle suppression (C-mitosis effect). The inhibition of blast transformation could conceiva-

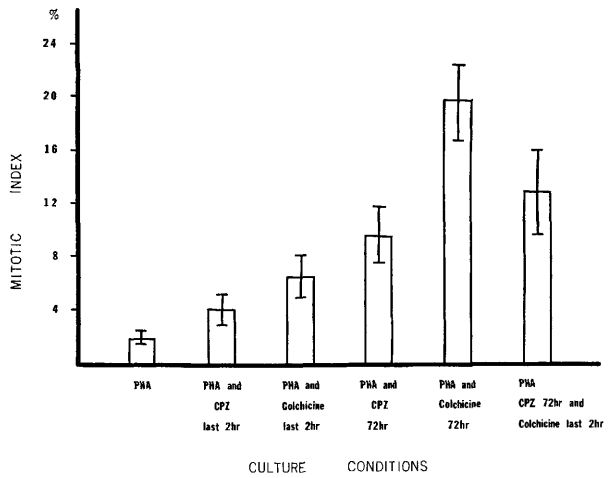

FIG. 4. Comparison of effects of CPZ (2×10^{-5} *M*) and colchicine on mitotic index of PHA-stimulated cultures.

TABLE II. Chromosome Aberrations of Human Leukocytes Treated with Various Concentrations of Meprobamate and Treated with 10^{-3} *M* Meprobamate Added at Various Culture Times.

	Conc (*M*) of meprobamate				Time of addition of meprobamate		
	Nontreated	10^{-5}	5×10^{-4}	10^{-4}	(hr): 0	18	48
Total cells with abnormal chromosomes in 500 cells examined	3	5	4	3	5	5	5
Abnormal cells (%)	0.6	1.0	0.8	0.6	1.0	1.0	1.0

bly be due to inhibition of enzymes reported by others (11, 14). Alternatively, an effect of CPZ on the cell membrane may be responsible. Spirtes and Guth (15) and Freeman and Spirtes (16) showed that CPZ (10^{-5} *M*) acts on the cell membrane of human erythrocytes and on rat mitochondria and affects the passive transport of water and ions across biological membranes. The inhibition of cell permeability may prevent the entrance of substances which normally stimulate transformation. This action may be akin to the membrane stabilizing effect on lysosomal enzyme release, a mechanism suggested as an explanation for the inhibition of blast transformation by prednisolone (17) and chloroquine (18).

Summary. The effects of chlorpromazine and meprobamate on chromosomes, blast transformation, and mitotic index were investigated in primary cultures of normal human leukocytes. Neither chlorpromazine nor meprobamate raised the rate of chromosome abberrations, when added at different times and in various concentrations. 2×10^{-5} *M* of CPZ decrease the number of blast cells but increased the mitotic index in 72-hour cultures if added during the first 18 hr. The increase in the mitotic index is apparently due to metaphase arrest (C-mitosis).

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