The melting profile of the chromatin DNA of healthy human lymphocytes when incubated in the patient's serum also was changed and consisted of a curve with a single diffuse maximum in the region of 85°C ($P < 0.01$), typical of the cell chromatin of patients with Down's syndrome (Fig. 1A, 2).

The investigation thus showed that the melting curve of DNA of the cell chromatin in the region between 78 and 85°C depends on the altered external environmental conditions, i.e., on the composition of the blood serum.

It can be concluded from the results of this comparative analysis of the structural (and, consequently, the functional) state of the chromatin of human lymphocytes that an altered composition of the blood serum causes reversible changes in chromatin structure, which result in a difference in the degree of dissociation of the nucleoprotein complex.

The most substantial differences between the melting curves of chromatin from normal and trisomic cells were observed within a narrow range of temperatures (78-85°C). This fact suggests that the main differences are connected with one chromatin fraction, structural modifications of which are manifested within precisely this temperature range.

The phenomena discovered can be regarded as important evidence which can help to explain the disturbance of synthesis of marker enzymes unconnected with the extra chromosome in trisomic cells [7]. The effect of the physiological environment on the structural and functional organization of the cell chromatin is thus clearly demonstrated.

LITERATURE CITED

1. V. N. Andrianov and D. M. Spitkovskii, Biofizika, 15, 14 (1970).
2. K. N. Fedorova and D. M. Spitkovskii, Byull. Éksp. Biol. Med., No. 6, 672 (1976).
3. K. N. Fedorova et al., Byull. Éksp. Biol. Med., No. 10, 97 (1974).
4. K. N. Fedorova et al., Humangenetik, 28, 183 (1975).
5. R. Rigler, Acta Physiol. Scand., 67, Suppl. 267, 1 (1966).
6. N. Ringertz and Z. Darzynkewicz, Exp. Cell Res., 62, 204 (1970).
7. W. Mellman and F. Osky, Lancet, 2, 674 (1964).

CYTOGENETIC EFFECTS OF CHLOROQUINE IN HUMAN LYMPHOCYTE CULTURES

M. A. Shalumashvili and Ya. A. Sigidin

UDC 615.283.926:547.831.4]015.4:612.6.05

Addition of chloroquine to a culture of human lymphocytes at the G_1 stage showed that the compound, in a concentration of 15 μg/ml, does not affect the level of chromosomal aberrations, but in concentrations of 60 and 100 μg/ml it suppresses mitotic activity of the cells virtually completely. By its actions on the G_2 stage chloroquine, in a concentration of 100 μg/ml, significantly increases the number of chromosomal aberrations, but in a concentration of 15 μg/ml it has no appreciable action.

KEY WORDS: chloroquine; chromosomal aberrations; lymphocyte cultures.

Experiments in vitro and clinical investigations in recent years have demonstrated the weak mutagenic action of two substances widely used in the treatment of rheumatism: aspirin [3, 4] and butadione [14]. Other substances used in rheumatology have not yet been studied from this standpoint. Among them, special attention

Clinical Pharmacological Group, Institute of Rheumatism, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician N. P. Dubinin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 7, pp. 879-881 (1976). Original article submitted January 14, 1976.

is due to chloroquine, which gives good results in the most stubborn forms of rheumatism, rheumatoid arthritis, all the other collagenoses, nephritis, and many chronic allergic syndromes. Moreover, this preparation is given continuously over long periods [1]: from 1 to 5 years or more. The question of its possible mutagenic effect is thus extremely important, more especially because there are concrete grounds for such a possibility.

The therapeutic action of chloroquine [7-chloro-4-(4-diethylaminoethyl-butylamino)-quinoline] is based on its cytostatic and immunodepressive effects, due to interaction between the compound and the nucleic acids of the cells [13]. It has been shown that chloroquine, by forming complexes with DNA and inhibiting DNA-polymerase [6, 7], disturbs processes of DNA synthesis [8] and, in particular, blocks the replication of coli-phage [15] and DNA-containing herpes simplex virus. This marked effect on DNA metabolism makes a cytogenetic analysis of the action of chloroquine essential. However, only one such investigation [12] has so far been carried out on patients with rheumatoid arthritis. The results showed that the total number of chromosomal aberrations in patients treated with a combination of chloroquine and aspirin was not significantly greater than in patients receiving aspirin alone. However, the number of unstable chromosomal aberrations (fragments and dicentrics) was significantly increased. However, the use of two preparations simultaneously in this case complicates the evaluation of the results.

Since no previous cytogenetic analysis has previously been undertaken during administration of chloroquine alone, it was decided to begin the study of this problem with such investigations in cultures of human lymphocytes.

EXPERIMENTAL METHOD

Blood cultures were prepared by the usual method [9]. Blood from healthy donors and patients with rheumatism under 35 years of age was used. Because of the well known cytostatic action of chloroquine, cultivation continued for 72 h. Chloroquine, in various concentrations (15, 60, and 100 μg/ml) was added at the G_1 (1.5 h after phytohemagglutinin stimulation) stage without washing off, and at the G_2 stage (4.5 h before fixation). Colchicine was added 3 h before fixation. Chromosomal aberrations were analyzed at the metaphase stage; altogether 1,252 metaphase plates were studied. All concentrations of chloroquine used in the culture medium were equivalent to its concentration in the patients' serum when used for therapeutic purposes.

EXPERIMENTAL RESULTS

After addition of chloroquine in concentrations of 60 and 100 μg/ml at the G_1 stage the four cultures contained only solitary metaphase plates. This may be due either to death of the cells or to a sharp decrease in their ability to proliferate under the influence of the doses of chloroquine specified above. Addition of chloroquine at the G_1 stage in a concentration of 15 μg/ml did not lead to a significant increase in the frequency of chromosomal aberrations: $2.5 \pm 0.3\%$ in the experimental series and $1.5 \pm 0.3\%$ in the control ($P > 0.05$).

When added at the G_2 stage in a concentration of 15 μg/ml, chloroquine likewise had no mutagenic action, but in a concentration of 100 μg/ml it led to a marked increase in the number of chromosomal aberrations: $5.8 \pm 1.4\%$ in the experimental series compared with $1.7 \pm 0.6\%$ in the control ($P > 0.05$). The frequency of aberrations in cultures of lymphocytes obtained from healthy donors and from pateints with rheumatic fever was the same.

To explain the mechanisms of the mutagenic action of chloroquine, data indicating that the binding of chloroquine with DNA leads to stabilization of the latter and prevents its denaturation [11] are important. In this connection an analogy between the action of chloroquine and the well-known effect of polyfunctional alkylating compounds can be postulated. Under the influence of those mutagenic factors, intermolecular cross linkages are formed in double-stranded DNA molecules (i.e., stabilization of the DNA also occurs), and as a result the likelihood of the appearance of chromosomal aberrations is increased [5,10].

All aberrations induced by chloroquine were of the single fragment type. The absence of aberrations of the exchange type and a marked increase in the number of gaps in the experimental series (5.4%) compared with the control (0.2%) will be noted.

These results points to the necessity for a special study of the mutagenic effect of chloroquine under clinical conditions.

LITERATURE CITED

1. T. I. Bibikova and Ya. A. Sigidin, The Treatment of Rheumatic Fever with Quinoline Preparations [in Russian], Moscow (1974).

2. V. D. Zhestyanikov, Repair and Radioresistance of the Cell [in Russian], Leningrad (1968).
3. N. M. Karaseva, Byull. Éksp. Biol. Med., No. 10, 100 (1974).
4. V. A. Nasonova, N. M. Karaseva, V. B. Khvatov, et al., Ter. Arkh. No. 3, 26 (1972).
5. B. M. Alberts, J. Molec. Biol., 32, 405 (1968).
6. J. L. Allison, R. L. O'Brien, and F. E. Hahn, Science, 149, 1111 (1965).
7. S. N. Cohen and K. L. Yielding, Arthr. Rheum., 7, 302 (1964).
8. H. Greiling, R. Kisters, and O. Vojtisek, Z. Rheumaforsch., 29, 146 (1970).
9. D. A. Hungerford, Stain Technol., 40, 333 (1965).
10. I. N. Iger and W. Szybalski, Proc. Nat. Acad. Sci. (USA), 50, 355 (1963).
11. N. B. Kurnick and I. E. Radcliffe, J. Lab. Clin. Med., 60, 669 (1962).
12. W. A. Neill, G. S. Panayi, and J. J. R. Duthie, Ann. Rheum. Dis., 32, 547 (1973).
13. F. S. Parker and J. L. Irvin, J. Biol. Chem., 199, 897 (1952).
14. A. C. Stevenson, J. Bedford, A. G. S. Hill, et al., Ann. Rheum. Dis., 30, 487 (1971).
15. K. L. Yielding, Clin. Res., 15, 72 (1967).