In Vitro Effect of Chloramphenicol on Chromosomes

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Bone marrow toxicity induced by chloramphenicol is of great hematologic importance. The occurrence of bone marrow aplasia, the development of paroxysmal nocturnal hemoglobinuria in the wake of marrow hypoplasia and the development of acute leukemia indicate the extent of the problems that can be encountered. Our previous observations, as well as studies by others, have shown the presence of nuclear and cytoplasmic vacuolations in hematopoietic cells of patients on prolonged chloramphenicol therapy. Following the cessation of therapy, the vacuoles gradually disappear. More recently we have found intrachromosomal vacuoles in patients treated with large doses of chloramphenicol. In addition, breaks, gaps and fragmentation, which apparently originated from the vacuoles, were also observed.

The present experiments were conducted to determine whether similar lesions could be produced in vitro by this drug.

Materials and Methods

Short-term (72-hour) cultures of white blood cells from normal individuals were performed according to the method of Moorhead et al. Chloramphenicol was added to the cultures six hours before harvesting, in amounts to produce a final concentration of 10, 25 or 40 μg/ml culture medium. Controls consisted of cultures to which no chloramphenicol was added, but to which the diluent used to dissolve the drug, was added. Following harvesting of the leukocytes, chromosomal preparations were obtained according to the above-mentioned method. Metaphases were examined under microscope and the abnormal ones were photographed for further analysis. All together, 23 control experiments were done and 30 were carried out with chloramphenicol, 10 of each with 10, 25 and 40 μg/ml. of medium, respectively. Additional controls consisted of five experiments with penicillin, eight units per ml.; five with 15 μg/ml. streptomycin; and five with 30 μg/ml. streptomycin/ml. Fifty metaphases were examined. In each culture the following chromosomal changes were looked for: breaks, gaps, fragments, secondary constrictions and intrachromosomal vacuoles. The results were expressed as percentage of metaphases with abnormalities.

Results

The following structural chromosomal abnormalities were encountered: gaps, breaks, fragments, secondary constrictions and intrachromosomal vacuoles. In one metaphase (10 μg/ml. chloramphenicol) two ring chromosomes were seen (Fig. 1).
The mean of metaphases with chromosomal changes among 1150 metaphases in the control group was 11.7 per cent. In the chloramphenicol containing cultures, the values were as follows: 10 µgm., 23.4 per cent; 25 µgm., 29.2 per cent; and 40 µgm. 35.2 per cent. The differences between control and chloramphenicol groups are statistically highly significant, (p less than 0.001). The mean of metaphases with changes in the penicillin group was 18.8 per cent; in the cultures incubated with 15 µgm. streptomycin, 15.6 per cent; and in cultures incubated with streptomycin, 30 µgm., 15.6 per cent. The p value in
these experiments was $0.001 < p < 0.01$ for penicillin and $0.01 < p < 0.02$ for streptomycin.

Intrachromosomal vacuoles were not seen in the control metaphases. They were present in 3.2 per cent of metaphases from cultures containing 10 µgm. chloramphenicol; in 1.6 per cent of metaphases from cultures incubated with 25 micrograms; and in 4.5 per cent of metaphases from cultures incubated with 40 µgm. chloramphenicol. Leukocytes incubated with penicillin and streptomycin (15 µgm./ml.) showed vacuoles in 0.8 per cent of metaphases. No vacuoles were present in chromosomes from cultures containing 30 µgm./ml. streptomycin.

**Discussion**

Abnormal chromosomes may result from inherited or environmental factors. Down's syndrome, in which a numerical abnormality is present (G trisomy); or Fanconi's anemia and Bloom's syndrome, in which structural changes are present, are examples of inherited lesions. Each of these disorders is associated with a high incidence of neoplasia, which is usually leukemia. Of the environmental factors leading to chromosome damage, X-rays have been extensively studied as a cause of neoplasia. Viruses, both carcinogenic and non-carcinogenic, are also known to interfere with chromosomal structure. It is worth noting that, although both adenovirus 12, which is carcinogenic, and adenovirus 2, which is not, produce chromosomal lesions, only adenovirus 12
(carcinogenic) leads to rearrangement of damaged chromosomes. In this respect it is similar, if not identical with, the effects of radiation, which also lead to rearrangement of damaged chromosomes.

Of special significance to this study are the findings that chemicals and antimetabolites cause chromosomal damage. Chromosomal lesions such as breaks, gaps and fragments, observed by Kiossoglou et al. in patients with pernicious anemia, indicate that the lack of a metabolite can also cause similar abnormalities. With Vitamin B₁₂ therapy and improvement of DNA metabolism, these changes were largely corrected. Similarly, inhibitors of DNA synthesis such as 5-fluorodeoxyuridine, deoxyadenosine, arabinosylcytosine, arabinosyladenine, and cytidine triphosphate also result in similar changes. Depletion of arginine in the culture medium has also been observed to lead to chromosomal breaks.

The present studies confirm our previous observations in patients that chloramphenicol may injure chromosomes, for they show that the addition of this drug to normal leukocyte cultures in concentrations comparable to in vivo therapeutic levels cause chromosomal damage. The degree of this damage increases with an increase of the level of the drug.

In in vitro cultures, a certain number of chromosomal changes will usually be present, but none or only a few are found in the direct bone marrow method of chromosomal studies. This is most likely due to the nonphysiologic, artificial environment to which dividing cells are exposed. In the present study 11.7 per cent of controls showed such changes, but the addition of chloramphenicol resulted in a highly statistically significant increase of such changes. Cultures containing 8 units penicillin/ml. of medium or 15 or 30 μgm. streptomycin/ml. of medium showed 18.8, 15.6, and 15.6 per cent of metaphases with chromosomal changes, respectively. These levels are above the levels of the control group, but below the levels obtained in the chloramphenicol group.

The nature of the biochemical effect of chloramphenicol on chromosomes is not clear. The effect of chloramphenicol on bacteria has been studied extensively. Levels of 10 μgm./ml. inhibit protein synthesis in sensitive bacteria. As a result, various enzymes are not synthesized. Chloramphenicol inhibits protein synthesis by interfering with the attachment of messenger RNA to ribosomes. DNA and RNA continue to be synthesized; however, Doudney noted that DNA synthesis can be inhibited if the antibiotic is added just prior to cell division. This inhibition is probably due to interference with the synthesis of enzymes involved in nucleic acid synthesis.

Although chloramphenicol readily inhibits protein synthesis in microbial systems, protein synthesis in mammalian cells is resistant to the drug. Weisberger et al., and Weisberger and Wolfe have shown that chloramphenicol can inhibit protein synthesis in cell-free mammalian systems as effectively as in microbial systems if template RNA is added to the mixture. Zelkowitz et al. disagree with this observation and state "the response of the protein synthesizing machinery in mammalian cells to chloramphenicol is different from that in bacteria."

How could interference with protein synthesis by chloramphenicol lead to chromosomal damage? The drug may interfere with chromatin-associated
proteins (histones), and thus expose chromosomal DNA to damage. Or, as stated before, DNA synthesizing enzymes may be inhibited, resulting in imperfect DNA formation. Finally, in some as yet unclear way, chloramphenicol may directly damage the chromosomal DNA itself.

Because of the short time during which the cells were observed, one would not expect to find morphologic evidence of chromosomal rearrangements. The presence of ring chromosomes in one of the metaphases is of interest because it may indicate that chromosome damage by chloramphenicol are liable to rearrangement. The development of new, abnormal cell lines after exposure to chloramphenicol may be of significance in the evolution of neoplasia.

SUMMARY

Leukocytes from normal individuals were cultured in media containing various concentrations of chloramphenicol. Chromosomes obtained from these cultures were studied for intrachromosomal vacuolation and various other structural changes such as breaks, gaps, fragmentations, etc.

Chromosomes from chloramphenicol containing cultures showed significant increase in abnormalities when compared with control preparations. Changes observed were very similar to those seen in patients receiving large doses of chloramphenicol.

REFERENCES

18. Rendi, R., and Ochs, S.: Effect of chloramphenicol on protein synthesis cell


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