Inhibition of Mitosis by Chloramphenicol in Phytohemagglutinin Stimulated Lymphocytes

By Anthony V. Piscotta and Cecelia DePrey

Various clinical and experimental studies have shown that chloramphenicol (CAP) affects cell division. The characteristic aplastic marrow that is seen when toxicity is fully expressed suggests a toxic effect leading to depletion of dividing cells. The effect of CAP on incorporation of P³² into phytohemagglutinin (PHA) stimulated lymphocytes was reported by McIntyre and Ebaugh, who found diminished specific activity of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and acid soluble fractions after incubation with this drug. Similar inhibition of cell division was reported in other experimental models, such as poliovirus-infected Hela cells, human carcinoma cells in the logarithmic phase of growth, and cultures of chlamydomonas. On buccal smear preparations Barr bodies were found to have a smaller dimension after a week of clinical treatment with CAP. Mitotic activity of human bone marrow cells, especially of the basophilic normoblasts, was diminished to a slight degree after clinical treatment with CAP.

When quiescent lymphocytes are exposed to PHA, they transform to actively dividing cells, having all the characteristics of blast forms. By adding colchicine shortly before the culture is terminated, mitosis is arrested at metaphase and mitotic figures accumulate. The effect of drugs on blast transformation and mitosis has been reported, including chlorpromazine, chloroquine, prednisolone and others. In this paper, our studies on the effect of chloramphenicol will be reported.

Materials and Methods

Human peripheral blood was heparinized and cultured by the phytohemagglutinin technic of Moorhead et al. Leukocytes were separated from whole blood by sedimenting erythrocytes with PHA and then incubating them in flat, sterile, screw-capped medicine bottles, lying on their side for 72 hours. Most cultures were prepared from 5 ml. of autologous serum, PHA, 15 ml. of culture medium TC 199, and 12 x 10⁶ leukocytes. In some cases, half these amounts were used.

The chloramphenicol solution was freshly prepared for each experiment to provide a
Table 1.—Effect of Chloramphenicol on Cell Division 2 Hours after Addition of Colchicine to 70-Hour PHA Stimulated Culture of Peripheral Blood in 43 Random People

<table>
<thead>
<tr>
<th></th>
<th>Mitoses/1000 Cells (± 1 S.D.)</th>
<th>Per cent Mitoses + Blasts (± 1 S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mitotic Index)</td>
<td></td>
</tr>
<tr>
<td>No Drug</td>
<td>34 ± 12</td>
<td>22 ± 10</td>
</tr>
<tr>
<td>CAP</td>
<td>11.7 ± 9.5</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>Diff.: No Drug—CAP</td>
<td>1.4 ± 8</td>
<td>78 ± 10</td>
</tr>
</tbody>
</table>

| t                 | <.001                         | <.001                                |
|                   |                                |                                      |
|                   |                                | 0.578                                |
|                   |                                | 1.123                                |

Results

The mitotic index (accumulation of mitoses/1000 cells, 2 hours after addition of colchicine to a 70-hour culture) and blast transformation (per cent mitoses plus blasts) were determined at hourly intervals following exposure to colchicine (Fig. 1). Subjects 1, 2, 6, 8, 9, 10, 11 and 12 showed a consistent lag in mitoses in CAP treated cultures for the entire 5-hour period of observation. Not all people showed depression of mitotic index (subjects 4, 5 and 7); one subject (#3) was not suppressed in the first 2 hours but mitosis lagged in the last 3 hours. The number of blast cells in all CAP treated cultures remained equal to the no drug controls (not illustrated).

Data obtained from the CAP sensitive patients are presented in Figure 2. Like the normals (Fig. 1), consistently fewer mitotic figures accumulated
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Fig. 1.—Effect of chloramphenicol (CAP) on rate of accumulation of mitoses after addition of colchicine to 70-hour PHA stimulated cultures of peripheral blood in 12 normal patients.

Fig. 2.—Effect of CAP on rate of accumulation of mitotic figures in 70-hour PHA stimulated culture of peripheral blood in two patients with CAP induced aplastic anemia.

following addition of colchicine in preparations incubated with CAP, as compared with no drug controls. However, possible differences from normals were seen in the CAP sensitive patients' preparations. For one thing, even without CAP, fewer mitoses accumulated in the patient's culture than in normals. In patient M.K., the per cent blast transformation was 87 per cent when the drug was omitted and 64 per cent when CAP was included in the cultures, whereas in patient F.S. per cent blast transformation was 89 per cent in the control and 84 per cent in the presence of CAP.

It was necessary to add CAP at the initiation of culture in order to inhibit mitosis (p. < .01) (Figs. 3A and 3B). Addition of CAP in 24 and 48 hours had
These studies show that chloramphenicol inhibits cell division but not blast transformation of PHA stimulated lymphocytes. Its action thus differs from drugs that suppress both, such as chlorpromazine and chloroquine. This selective effect probably localizes the CAP effect to events that occur around the G-2 phase of cell division, which prevents cells from entering the M phase. Our data do not clarify the precise mechanism of CAP suppression of mitosis. The dominant effect of CAP takes place during the first 24 hours of incubation, although a definite but less marked inhibition is seen when the drug is added in 24 or 48 hours.

Not all the events that lead to blast transformation and cell division are known. During the first 24 hours that lymphocytes are exposed to PHA, RNA is synthesized. At some time later, preparation for protein synthesis involves the deposition of messenger RNA on the ribosomes. These events seem to coincide with the time at which peak effect of CAP is observed in PHA stimulated cultures. Studies in cell free systems seem to show that CAP...
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Table 2.—Effect of Phenylalanine on CAP Depression of Mitotic Index

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No Drug</th>
<th>CAP</th>
<th>CAP + 0AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>X</td>
<td>32</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>S</td>
<td>9.5</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3.—Role of Colchicine in Accumulation of Mitoses in PHA Stimulated Peripheral Blood Cultures

<table>
<thead>
<tr>
<th></th>
<th>Number of Mitotic Figures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 Hours</td>
</tr>
<tr>
<td>Colchicine</td>
<td>56</td>
</tr>
<tr>
<td>No colchicine</td>
<td>18</td>
</tr>
<tr>
<td>Colchicine + CAP</td>
<td>—</td>
</tr>
<tr>
<td>No colchicine + CAP</td>
<td>—</td>
</tr>
</tbody>
</table>

inhibits binding of RNA to ribosomes. Nevertheless, this matter is as yet unsettled because inhibition of RNA binding to ribosomes may affect blast transformation as much as mitosis.

In PHA stimulated cultures, synthesis of DNA begins some time later and reaches its peak in 48 to 72 hours as the cells begin to divide. It seems likely that CAP does not directly affect DNA synthesis. For one thing, CAP does not alter labeling index of marrow cells incubated with H³ thymidine. Furthermore, incorporation of H³ thymidine or H³ thymidine triphosphate into DNA in a cell-free system is not affected when CAP is included in the incubation mixture. Lack of demonstrable restraint of blast transformation in lymphocyte cultures also suggests that neither synthesis of DNA nor of the involved enzymes are influenced by this antibiotic. Despite this, cells treated with CAP do not divide well. Krause and Plaut pointed out that when CAP treated onion root tips were incubated with H³ thymidine, radioautographic preparations showed significantly higher grain counts per cell but lower per cent labeled cells than control preparations. When cell division is prevented, distribution of radioactive material to the daughter cells does not occur, resulting in higher mean grain counts but fewer labeled cells. In McIntyre and Ebaugh's study, the net effect of CAP may have resulted in a smaller number of dividing cells capable of incorporating P³² into DNA.

A large body of work has related the effect of CAP to RNA and protein synthesis. In high concentrations, CAP diminished incorporation of H³ uridine into marrow cells in vitro. In bacteria exposed to CAP, an unstable RNA fraction is produced that is degraded readily and cannot attach to ribosomes. In mammalian as well as bacterial systems, CAP inhibited deposition of messenger RNA on the ribosome and suppressed protein synthesis at this
locus. As a possible consequence, CAP is believed to prevent formation of proteins required for cell division, possibly mitotic spindle protein. Taylor has provided some direct observations in support of this concept. When CAP was added to newt myocardial fibers in tissue culture in early prophase, it prevented the formation of a mitotic spindle of sufficient size to function efficiently. If mitotic spindle protein manufacture is compromised by CAP, the cells cannot divide, but continue to synthesize DNA.

According to Yunis and Harrington, CAP suppressed incorporation of C\textsuperscript{14} formate into DNA and RNA of marrow cells from normals, in the rather high concentration of 250 \( \mu \text{g.} / \text{ml.} \). When marrow was obtained from CAP sensitive patients, a concentration of 50 \( \mu \text{g.} / \text{ml.} \) of CAP was sufficient to inhibit incorporation of formate into nucleic acids. In our studies, about 60 \( \mu \text{g.} / \text{ml.} \) suppressed mitosis in PHA stimulated lymphocytes from normals. This amount is high but approaches feasible pharmacologic levels. Lorian pointed out that 1 Gm. of CAP was sufficient to provide a blood level of 8 to 25 \( \mu \text{g.} / \text{ml.} \), 2 hours after a single administration.

The demonstration of an individual cellular defect in a CAP sensitive patient must be interpreted with caution, because any deviation from normal might result from irreversible disease or from the effects of therapy. For example, prednisone may inhibit PHA stimulated mitosis in vitro and may have a similar effect when administered clinically. Furthermore, limited accumulation of mitosis in CAP sensitive patients may be entirely coincidental and many more studies must be performed in order to establish possible significance.

Finally, the question of the effect of phenylalanine on CAP induced depression was examined. It was reported that infants on a phenylalanine deficient diet develop cytoplasmic vacuoles in their immature bone marrow cells similar to those produced by toxicity to CAP. Treatment with phenylalanine caused these vacuoles to disappear. Unfortunately, simultaneous treatment of patients with phenylalanine and CAP did not protect them from myelotoxicity. Likewise in our culture system, phenylalanine in vitro did not affect suppression of mitosis induced by CAP.

**SUMMARY AND CONCLUSIONS**

1. When lymphocytes from human peripheral blood are treated with phytohemagglutinin, they undergo transformation to blast cells and then divide.
2. When chloramphenicol was added to the culture at the beginning of the experiment, mitosis was delayed but blast transformation was not affected.
3. CAP suppressed mitosis less markedly when added to the culture at 24 and 48 hours and more markedly when added daily.
4. Mitotic index was suppressed in two patients who had aplastic anemia due to CAP, in a manner similar to that observed in presumably normal people.
5. Possible reasons for this drug effect on cell division are discussed.

**SUMMARIO IN INTERLINGUA**

1. Quando lymphocytos ab human sanguine peripheric es tractate con phytohemagglutinina, illos se transforma in blastocytos e alora se divide.
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2. Quando chloramphenicol esseva addite a cultura al comenciamento del experimento, le mitose esseva retardate sed le transformation blastocytic non esseva alterate.

3. Chloramphenicol supprimeva le mitose minus marcatemente quando illo esseva addite al cultura post 24 e 48 horas e plus marcatemente quando illo esseva addite omne die.

4. Le indice mitotic esseva supprimite in duo patientes qui habeva anemia aplastic in consequentia de chloramphenicol in un maniera simile a ihlo observate in apparentemente normal subjectos.

5. Le possibile explicationes de iste effecto pharmacologic in le division cellular es vommentate.

REFERENCES


16. Pisciotta, A. V.: The effect of chloramphenicol on DNA kinase and poly-
merase activity. Unpublished data.


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