SUMMARY

Streptovitacin A (a protein synthesis inhibitor) partially protected DON cells from the lethality of l-fl-D-arabino-furanosylcytosine (cytarabine), hydroxyurea, and 5-hydroxy-2-formylpyridine thiosemicarbazone (DNA synthesis inhibitors and S-phase-specific agents). Our results suggest that the cells in S were not protected by streptovitacin A while the non-S-phase cells were protected.

INTRODUCTION

Proliferating crypt epithelial cells are killed when they are exposed in vivo to the DNA synthesis inhibitor, ara-C (11). However, compounds such as cycloheximide or tenuazonic acid, which simultaneously inhibit both protein and DNA synthesis, do not kill crypt epithelial cells. Furthermore, these cells can be protected from the cytotoxicity of ara-C by simultaneous exposure to cycloheximide or tenuazonic acid (11). These findings in the intact animal suggest that (a) continued protein synthesis is necessary in order that inhibition of DNA synthesis will result in cell death, and (b) when both protein and DNA synthesis are simultaneously inhibited, the cells are protected from the lethal effects of the DNA synthesis inhibitor (7, 11). These results support the concept of unbalanced growth or thymineless death presented by Cohen and Barner (5) for bacteria and subsequently verified for eukaryotic cells (13).

The experiments of Lieberman et al. (11) with crypt epithelial cells could not be made quantitative since changes in cell morphology were the only criteria for cell death. Our studies with DON cells in culture were undertaken to obtain quantitative data on the antagonism between DNA and protein synthesis inhibitors.

MATERIALS AND METHODS

Cell Culture

DON cells from a Chinese hamster fibroblast line (ATCC, CCL16), were grown at 37° in McCoy’s 5A medium modified by the addition of lactalbumin hydrolysate (0.8 g/liter), fetal calf serum (200 ml/liter), and Panmede (ox-liver extract prepared by Paines and Pyrne, Ltd., Greenford, England; 0.5 g/liter). The medium was obtained from the Grand Island Biological Company, Grand Island, N. Y. The cells were grown in 8-oz prescription bottles planted with about 2 × 10⁶ cells in 25 ml of medium and were maintained in logarithmic growth by subculture every 2 days. With renewal of the medium every 2 days, an 8-oz bottle would support logarithmic cell growth, with a generation time of 10 to 12 hr, until there were about 10⁸ cells/bottle. For subculturing, the cell monolayer was detached from glass by treatment with a 0.1% trypsin solution; the cells were then dispersed in medium, and an aliquot was planted in bottles.

Synchronous DON Cells

A logarithmically growing culture was exposed to Colcemid and a population of mitotic cells was selectively harvested. The mitotic cells, which constituted between 85 and 95% of the harvested cells, were used to start the synchronous culture. The method has been described in detail previously (2).

Determination of Percentage of Cell Survival after Exposure of DON Cells to Drug

After exposure to drug, the supernatant medium was poured off, and the cells were detached with trypsin and resuspended in medium at 37°. The cells were diluted in medium at 37°, and 2 ml of cells were planted in plastic Petri plates (Linbro Chemical Co., New Haven, Conn.) to give 10 to 100 colonies per plate. Twelve plates were planted for each sample. After incubation for 7 to 8 days in an atmosphere of 8% CO₂:92% air at 37°, the medium was poured off, and the colonies were stained with 0.2% methylene blue in 70% ethanol. The plating efficiencies were about 50% for synchronous cells and 70% for asynchronous cells. For calculation of the percentage of survivals, the control (no drug treatment) samples were normalized to 100% survival. In these experiments, the coefficient of variation in determining cell survival was about 15%, the coefficient of variation being the standard deviation expressed as a percentage of the mean. Thus, if the percentages of survival of 2 different samples were 50 and 30%, respectively, then they would be statistically significantly different at the 95%
DNA Synthesis Inhibition and Cell Death

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg/ml)</th>
<th>DNA % inhibition</th>
<th>RNA % inhibition</th>
<th>Protein % inhibition</th>
</tr>
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<tbody>
<tr>
<td>ara-C</td>
<td>100</td>
<td>92</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>5-HP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HU</td>
<td>300</td>
<td>97</td>
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<td>23</td>
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<td>22</td>
<td>73</td>
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<td>4</td>
<td>10</td>
<td>32</td>
</tr>
</tbody>
</table>

The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine (cytarabine, NSC 63878); HU, hydroxyurea; 5-HP, 5-hydroxy-2-formylpyridine thiosemicarbazone (NSC 107392); HSA-TdR-3H, high-specific-activity thymidine-3H (6.7 Ci/mmole).}

RESULTS

Effect on Macromolecule Synthesis. The inhibition of macromolecule synthesis by ara-C, 5-HP, HU, and streptovitacin A is shown in Table 1. The results show that ara-C, 5-HP, and HU maximally inhibited DNA synthesis while streptovitacin A markedly inhibited both protein and DNA synthesis. Under these conditions the uptake of TdR-3H or valine-14C into the acid-soluble pool of the cells was not affected by the drugs in question.

Effect of Combination of Streptovitacin A with Ara-C, HU, or 5-HP on Cell Survival. Chart 1 shows the effect of ara-C and streptovitacin A, singly or in combination, on DON cell survival. The results obtained are compared with the survival of cells exposed to HSA-TdR-3H (10 µCi/ml, 6.7 Ci/mmole). TdR-3H kills S-phase cells subsequent to its incorporation into DNA (4) and does not affect the passage of cells into S at the concentration used (3).

The results indicate the following. (a) During the 1st hr, HSA-TdR-3H killed 63% of the cells. ara-C alone, or in combination with streptovitacin A, killed 68% of the cells in the 1st 2 hr. These were presumably the cells in S phase, and this point is considered further in the discussion section. (b) Between 2 and 8 hr, the rate of cell kill with ara-C alone was much lower than that with HSA-TdR-3H such that at the end of 8 hr only 0.5% of the cells exposed to HSA-TdR-3H survived compared to 8% survival of cells exposed to ara-C alone. These results suggest that ara-C delayed the entry of non-S-phase cells into the drug-sensitive S phase. A similar plateau in the survival curve of L1210 cells (3) and L-cells (8) exposed to ara-C has been reported and was explained as indicating a reduced rate of flow of G1 cells into the drug-sensitive S phase. After 8 hr, a rapid rate of cell kill was observed with ara-C alone. The cells exposed to ara-C alone continued to die during...
the total period (12 hr) of exposure so that, by the end of 12 hr, only 0.7% of the cells survived. In contrast, cells exposed to ara-C plus streptovitacin A (both added at 0 time) did not die after the 1st 2-hr exposure, so that by the end of 12 hr 28% of the cells survived. These results clearly show that, although streptovitacin A did not protect the cells killed by ara-C during the 1st 2 hr, the remaining viable cells were protected from ara-C cytotoxicity by streptovitacin A.

The protective action of streptovitacin A could be seen when it was added even 4 hr after ara-C. Thus when streptovitacin A was added 4 hr after ara-C, the cell survival at 12 hr was 13% with the combination, compared with 0.7% with ara-C alone.

When streptovitacin A was added 1 or 4 hr before ara-C, the cell survival was the same as that obtained when the 2 drugs were added together.

The effect of ara-C, streptovitacin A, and the combination of the 2 drugs on DON cells in S phase was determined. Mitotic DON cells were incubated for 4 hr so that, at that time, 85 to 90% of the cells were in S phase. The S phase cells were treated for 1 hr with either ara-C (100 μg/ml), streptovitacin A (0.2 μg/ml), or the combination of the 2 drugs. Streptovitacin A killed only 6% of the cells, ara-C killed 54%, and the combination killed 54%. Therefore, these results show that streptovitacin A did not protect S phase cells from the cytotoxicity of ara-C.

Charts 2 and 3 show the effect of 5-HP and HU singly or in combination with streptovitacin A on DON cell survival. Streptovitacin and the DNA synthesis inhibitors were added together at Time 0. The results show (Chart 2) that during the 1st 5 hr, the same percentage of cells were killed by 5-HP and 5-HP plus streptovitacin. During the next 19 hr, 5-HP killed cells at a rapid rate so that only 0.2% of the cells survived, compared with 7% cell survival with 5-HP plus streptovitacin. Similar results were also obtained with HU.

Sterptovitacin A also protected L1210 cells from the cytotoxicity of ara-C. The same percentage (20 to 30%) of the L1210 cells survived after the 1st 2 hr of exposure to ara-C (10 μg/ml) or of ara-C (10 μg/ml) plus streptovitacin A (0.025 μg/ml). However, after 10 hr of exposure to ara-C, only 2.6% of the cells survived, compared with 18% survival for cells exposed to ara-C plus streptovitacin A.

DISCUSSION

Our results show, on the basis of precursor incorporation, that ara-C, HU, and 5-HP inhibited DNA synthesis, while streptovitacin A inhibited both DNA and protein synthesis. However, changes in pool sizes or in degradation rates could be affected by the administration of these drugs.

We showed that streptovitacin A (a protein synthesis inhibitor) protected both DON and L1210 cells from the cytotoxic effects of long-term exposure to several DNA synthesis inhibitors. Thus, after 12 hr of exposure to ara-C alone, only 0.7% of the DON cells survived compared to 28% survival of cells exposed to ara-C plus streptovitacin A.

In order to understand the mechanism of the protective action of streptovitacin A, it is necessary to look at the cellkill kinetics of ara-C, HU, and 5-HP. Previous studies with synchronous DON cells showed that ara-C, HU, and 5-HP were specifically cytotoxic to cells in S (2). With S-phase-specific agents such as ara-C, HU, or 5-HP, cells in S at the
time of drug addition are killed within a short period of time (1). The remaining viable cells in G₂, M, and G₁ progress normally through the cell cycle until they reach and accumulate at the G₁-S boundary (1, 3, 8, 14). We previously showed, with both asynchronous L1210 cells and synchronous DON cells, that ara-C, HU, and 5-HP delayed the entry of non-S-phase cells into the drug-sensitive S phase (3). Some of these cells, which are blocked at the G₁-S boundary, may flow slowly into S and are killed, while the rest die because of unbalanced growth.

The present study suggests the following. (a) During the 1st 2 hr, HU, ara-C, and 5-HP killed about 60 to 70% of the cells, which are most likely the cells in S. This assumption is based on the facts that, on short exposure, these agents specifically kill S-phase cells and that, in an asynchronous DON cell culture, about 60 to 70% are in S, i.e., they are labeled by pulse exposure to TdR-³H and are killed within 1 hr by HSA-TdR-³H (Chart I). (b) When streptovitacin A was added, together with the DNA synthesis inhibitors, the percentage cells killed in the 1st 2 hr were the same with or without streptovitacin A. This suggested that cells in S were immediately killed by the DNA synthesis inhibitors and were not protected by streptovitacin A. This suggestion was proven to be true, since streptovitacin A did not protect S-phase cells in a synchronous culture from the cytotoxicity of ara-C. (c) DON cells exposed to ara-C plus streptovitacin A were not killed after the 1st 2 hr. This indicated that streptovitacin A protected, at least partially, the non-S-phase cells from the lethality of ara-C. Whether this protection was exerted by preventing the cells accumulated at the G₁-S boundary from progressing into S or by preventing unbalanced growth from occurring cannot be decided from these experiments. Kim et al. (10) showed that unbalanced growth does occur in HeLa cells exposed to ara-C. In their experiments, synchronized HeLa cells at the G₁-S boundary, when exposed to ara-C, stopped DNA synthesis but continued RNA and protein synthesis. Since S-phase cells were not protected from ara-C toxicity by streptovitacin A, we are not concerned here with the progression of S-phase cells through the cell cycle or with unbalanced growth in S-phase cells.

Whereas the protective action of streptovitacin A was evident by 5 hr in the cells exposed to ara-C, it was not evident until after 8 hr in the cells exposed to HU. The reason for this difference in response is not clear at present.

Previous studies have shown that both in vivo and in vitro cells can be protected from the lethality of DNA synthesis inhibitors by treatment with protein synthesis inhibitors. Lieberman et al. (11) reported that inhibition of protein synthesis to 75% or more (but not less) by cycloheximide and tenuazonic acid will also prevent cell damage induced by ara-C in crypt epithelial cells. Djordjevic and Kim (6) reported that treatment of HeLa cells in culture with streptovitacin A (a protein synthesis inhibitor) increased the survival of cells treated with mitomycin C (a DNA-binding agent). These studies indicate that both in vivo and in vitro cells can be protected from the lethality of DNA synthesis inhibitors by treatment with protein synthesis inhibitors.

Lieberman et al. (11) suggested that at least 75% inhibition of protein synthesis was necessary in order that cycloheximide may protect crypt epithelial cells from the lethality of ara-C. We found that 0.05 µg streptovitacin A per ml (~30% protein synthesis inhibition) was as effective as 0.2 µg of the drug per ml (~70% protein synthesis inhibition) in protecting from the lethality of ara-C. The difference between our findings and those of Lieberman could be attributed to the difference between in vivo and in vitro systems and to the difference between cycloheximide and streptovitacin A (acetoxy-cycloheximide).

Lieberman et al. (11) showed that the protective effect is tissue specific in that cycloheximide protected epithelial cells of the intestinal crypts, but not the neighboring lymphoid tissue, from ara-C. However, tissue specificity was lost in vitro; both DON cells (fibroblast) and L1210 cells (lymphocytes) were protected from ara-C by streptovitacin A.

REFERENCES

Antagonism between DNA Synthesis Inhibitors and Protein Synthesis Inhibitors in Mammalian Cell Cultures

Bijoy K. Bhuyan and Terence J. Fraser

Cancer Res 1974;34:778-782.

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