Differential Cytotoxic Effect of Gossypol on Human Melanoma, Colon Carcinoma, and Other Tissue Culture Cell Lines

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ABSTRACT

Gossypol, a yellow pigment from the cotton plant (Gossypium) was found to have anti-tumor cell effects against several tumor cell lines grown in tissue culture. Most sensitive to the drug were melanoma and colon carcinoma cells. After 24 hr of treatment with 10 ^M gossypol, over 90% of these cells were killed. A rough correlation existed between very rapidly growing cells and their sensitivity to the drug. For example, slow growing cells, such as normal embryonic lung fibroblasts and mammary adenocarcinoma, were least sensitive, requiring over 30 ^M gossypol in order to kill 90% of cells after 48 hr of treatment. These results indicate that gossypol has differential cytotoxic effects against certain tumor cell types, such as melanoma and colon carcinoma, and suggest that it might be of potential therapeutic value.

The drug appears to act as a metabolic poison rather than as an agent interfering with DNA synthesis at the concentrations used in the study. Cells sensitive to the drug contain the more cathodic forms of lactate dehydrogenase, lactate dehydrogenase isoenzymes. For example, gossypol has a high chemical reactivity and may impair spermatogenesis by a number of mechanisms (3). Gossypol is a strong antioxidant, chelates metal ions, and forms complexes with proteins and amino acids (1). For example, gossypol exhibited such a broad spectrum of reactivity especially against metabolic enzymes such as LDH, we reasoned that the drug should be particularly effective in killing tumor cells that relied heavily on anaerobic metabolic pathways for production of energy. Such cells should be killed differentially. To test this hypothesis, we treated normal and various tumor cell lines with gossypol. The following report summarizes the results of our study.

INTRODUCTION

Gossypol, a yellow pigment from the cotton plant (Gossypium), was first identified in China as a possible antifertility agent (10). The drug interferes with spermatogenesis, exerting a specific and reversible effect on developing sperm (9). The mode of action of gossypol has been of considerable recent interest. Gossypol has a high chemical reactivity and may impair spermatogenesis by a number of mechanisms (3). Gossypol is a strong antioxidant, chelates metal ions, and forms complexes with proteins and amino acids (1). For example, gossypol has been shown to chelate zinc (11), bind arginine and lysine (4), and bind and inactivate enzymes essential for oxidative phosphorylation (2). Recently, it has been shown that gossypol specifically inactivates LDH^X, an isoenzyme of LDH found in sperm (8).

Since gossypol exhibited such a broad spectrum of reactivity especially against metabolic enzymes such as LDH, we reasoned that the drug should be particularly effective in killing tumor cells that relied heavily on anaerobic metabolic pathways for production of energy. Such cells should be killed differentially. To test this hypothesis, we treated normal and various tumor cell lines with gossypol. The following report summarizes the results of our study.

MATERIALS AND METHODS

Effect of Gossypol on Different Human Cell Lines. Various human cell lines were grown in the presence of different concentrations of gossypol. After 24 hr, cell numbers were estimated from adherent cells. Table 1 summarizes the results. Human melanoma cell lines were most sensitive, showing 50% reduction in cell number relative to controls after treatment of the cells with gossypol in the concentration range of 3 to 7 ^M. The other cell lines could be grouped in the following order from most to least sensitive: colon carcinoma; fibrosarcoma; promyelocytic leukemia; mammary adenocarcinoma; T-lymphoblastoma; normal embryonic lung fibroblasts; and erythroleukemia. Chart 1 illustrates the differential sensitivity of some of the cell lines tested. The gossypol dose-response curve of WM9 is sharp at 10 ^M; nearly all cells have been killed after 24 hr. In contrast, WI38 and HT3 still show about 70% viability under the same conditions. Figure 1 illustrates the morphology of treated cells and control cells. After 50 hr in the presence of 10 ^M gossypol, WI38 cells show little morphological change in comparison to control cultures. In sharp contrast, treated WM9 cells are sparse and rounded and contain numerous vacuoles. These results indicate that gossypol has profound toxic effect on certain cell types, such as melanoma, and that the same effect occurs for other lines but at significantly higher gossypol concentrations.

1 This work was supported by Department of Health and Human Service Grant HL 28149 and C. N. R. Progetto Finalizzato, "Controllo della crescita neoepilepsica."
2 To whom requests for reprints should be addressed.
3 The abbreviation used is: LDH, lactate dehydrogenase.
Effect of Gossypol on Growth Rate. We attempted to determine if gossypol was more toxic on rapidly growing cultures. The experiment illustrated in Chart 2 shows that SW407 and WI38, in the presence of 30, 10, and 5 μM gossypol, suffer similar declines of growth rate. However, the growth rate of WM9 cells, which appear to be the fastest under these conditions, is much more severely affected, even at gossypol concentrations of 3 to 5 μM, which barely or slightly affect the growth rate of SW407 and WI38 cells. A correlation between growth rate and sensitivity of gossypol is, however, not absolute, because K562 cells, which show a high rate of growth, are not affected by these concentrations of drug (data not shown). Therefore, it would appear that rapid growth rate is not an absolute characteristic of cells sensitive to gossypol.

Mode of Action of Gossypol. We attempted to correlate the sensitivity of the cell line to gossypol with a specific metabolic characteristic of the cell line. The mode of action of the drug appeared to involve the block of the metabolism of the cell, since autoradiographic analysis revealed that the percentage of DNA-synthesizing cells, after a 2-hr pulse with [3H]thymidine, did not vary between control and gossypol-treated WM9 cells, even at concentrations which killed more than 90% of cells. These results were consistent with a rapid death of most cells, suggesting some block in energy production. We next examined the LDH isoenzyme pattern of sensitive and nonsensitive cells, reasoning that, since gossypol inhibits a specific LDH isoenzyme in sperm selectively (8), it may have similar effects on tumor cell lines. Fig. 2 shows that sensitive cells are enriched in cathodic forms of LDH and almost completely lack the anodic forms of LDH. On the contrary, resistant cell lines contain high levels of the anodic isoenzymes of LDH.

DISCUSSION

We have shown that gossypol, a yellow pigment extracted from cotton seeds, has in vitro cytotoxic effects against numerous cell lines. Melanoma and colon carcinoma appear to be the most sensitive to the drug, showing cytotoxic effects to as little as 5 μM of gossypol. Other cell lines, notably normal fibroblasts and certain slow growing tumors such as mammary adenocarcinoma, are sensitive to the drug at concentrations above 20 μM.

The drug appears to act through a mechanism that does not involve inhibition of DNA synthesis, since autoradiography of treated cultures showed that the surviving cells continue to synthesize DNA even when most of the population has already been killed. Therefore, it seems probable that the drug exerts its effect through an inhibition of the metabolism of the cell. Preliminary results showed that LDH isoenzyme patterns of sensitive cells contained more cationic LDH forms, suggesting that the drug may interfere with lactate production in the rapidly growing cultures by inhibiting these enzymes. This is a tempting hypothesis consistent with the postulates of Otto Warburg (13), who showed that tumor cells depended more heavily on anaerobic glycolysis for energy production. However, a detailed kinetic study on the inhibitory effects of gossypol on each of the isozymes of LDH must be performed in order to consider this a viable mechanism. In view of the many possible reactions that gossypol can have with proteins (1, 2, 4, 11), it is probable that gossypol exerts its cytotoxic effects through numerous mechanisms (see Chart 3 for structure).

Despite the high toxic effects of gossypol in tissue culture, the...
lethal dose in rats is high (2400 mg/kg body weight) (6), suggesting that gossypol has a low toxic effect against normal tissue in vivo. The low toxicity of gossypol in vivo coupled with its specific effect against certain tumor cell lines, such as melanoma, makes this drug a potentially useful anticancer agent. Further work must be done to determine its in vivo effect on tumors.

REFERENCES

Fig. 1. Effect of gossypol on cell morphology. Cells were grown for 50 hr in the presence of 10 μM gossypol. Phase-contrast microscopy, × 100.

Fig. 2. LDH isoenzyme patterns of various cell lines. Approximately 5 x 10^7 cells of each cell line were harvested, washed in phosphate-buffered saline, and resuspended in 100 μl of phosphate-buffered saline. The cell suspension was sonicated briefly and centrifuged at 10,000 x g for 2 min. Approximately 1 μl of each cell lysate was subjected to agarose gel electrophoresis for 20 min at 15°, 340 V, and 100-ms constant current. The gel was stained with LDH isoenzyme stain reagent (Sigma) for 1 hr at 37°, fixed in 10% acetic acid, washed in H2O, dried, and photographed. Lane 1, WI38; Lane 2, HT3; Lane 3, HT 1080; Lane 4, WM9; Lane 5, WM56; Lane 6, WM164; Lane 7, SW1116; Lane 8, SW 1084; and Lane 9, WI38.
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