Cancer Chemother Pharmacol (1985) 15: 20–25

Cancer Chemotherapy and Pharmacology

Antitumor effects of gossypol on murine tumors

Potu N. Rao[1], Yong-chao Wang[1], Eva Lotzova[2], Abbas A. Khan[2], Srinivasu P. Rao[1], and L. Clifton Stephens[3]

Departments of [1] Chemotherapy Research, [2] General Surgery, and
[3] Veterinary Medicine, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, TX 77030, USA

Summary. Since the male antifertility drug, gossypol, was shown to be a specific inhibitor of DNA synthesis at moderately low doses in cultured cells, its antitumor potential has been evaluated in three murine tumor models. The effects of gossypol on tumor growth and the survival of 10- to 12-week-old BDF_1 mice bearing mouse mammary adenocarcinoma 755 (Ca 755) or P388 or L1210 leukemias, all injected IP, were measured. At an optimum dose of 0.5 mg/mouse given as a single injection at 2 days (48 h) after the inoculation of 10^5 Ca 755 tumor cells, gossypol rendered 66% of the mice free of tumor cells, whereas the remaining 34% died of drug toxicity. The survival rate decreased sharply at doses on either side of the optimum. At suboptimal doses a major proportion of the tumor-bearing mice died of tumor, whereas at higher doses all the animals died of drug toxicity. In other words, the effective dose range of gossypol was rather narrow. The rapidly proliferating mouse leukemias, P388 and L1210, failed to respond to gossypol. Histopathological studies of various organs in the gossypol-treated mice revealed no consistent lesions that could give an indication of organ-specific toxicity of gossypol. The reduction in the myeloid series in the bone marrow of gossypol-treated mice may have been due to depletion rather than direct toxic effect. Further studies are essential to evaluate this compound with regard to its antitumor activity in other murine models.

Introduction

Since the publication of reports from China [2] that gossypol, a phenolic compound extracted from cotton seed and plant parts, has antifertility properties, a number of investigators have examined the effects of this compound in greater detail. Gossypol has been shown to reduce the mitotic index in phytohemagglutinin-stimulated human peripheral blood lymphocytes [17], even though it does not appear to be a mutagen according to the Ames test [4] and the sperm head abnormality assay in mice [10]. Several enzymes, including various dehydrogenases, ATPase, and other enzymes involved in mitochondrial oxidative phosphorylation, have been reported to be inhibited by gossypol [1, 5, 11, 13]. Ye et al. [19] showed that treatment of Chinese hamster ovary (CHO) cells and human lymphocytes with gossypol caused no increase in chromosome breakage or polyploidy, but reduced the mitotic index, the cell viability, and the rates of synthesis of DNA, RNA, and protein.

Studies from our laboratory have indicated that gossypol, at a concentration of 10 μg/ml, is a specific inhibitor of DNA synthesis in CHO and HeLa cells [18]. At this concentration it has no effect on the rate of RNA and protein synthesis. Gossypol-induced cytotoxic effects that are not dose dependent, i.e. a decrease in growth rate and plating efficiency of cultured cells, are observed only when a critical concentration is achieved. These observations have prompted us to examine gossypol as a potential antitumor agent. We investigated the effect of gossypol on three murine tumor models. In this article we report that gossypol at an optimum dose (0.5 mg/mouse) cured about 60% of mice bearing adenocarcinoma 755 (Ca 755) but did not increase the life span of mice inoculated with P388 or L1210 leukemia cells.

Material and methods

Mice Eight- to ten-week-old (C57BL/6 × DBA/2) F_1 (BDF_1) female mice were obtained from Cumberland View Farms, Clinton, Tenn and were acclimatized for 2 weeks to recover from shipping trauma. At the time of the experiment the mice were 10–12 weeks old with an average body weight of 20 g. Usually mice were housed eight per cage and fed standard laboratory chow. For these experiments each treatment group of six mice was put in a separate cage.

Tumor cells

Ca 755. This tumor was originally described by Bagg and Jacksen [3] as an adenocarcinoma of the breast in a C57 BL mouse. The kinetic parameters and therapeutic response of Ca 755 maintained as a solid tumor has been studied by Laster et al. [8]. For the present study Ca 755 has been maintained in Dr Lotzova's laboratory by in vivo IP passage of 2×10^6 cells every 12–15 days in BDF_1 hybrid mice, as described elsewhere [19].

For testing the antitumor effects of gossypol, 10^5 Ca 755 cells were injected IP. Six tumor-bearing mice were tested at each dose of gossypol: 0.4, 0.5, 0.6, 0.8 mg/mouse, given as a single or split doses. Control mice each received, 0.5 ml saline IP.

Offprint requests to: P. N. Rao

Mouse leukemias P388 and L1210. P388 lymphocytic leukemia and L1210 lymphoid leukemia cells were kindly provided by Dr William Plunkett, Department of Chemotherapy Research, M.D. Anderson Hospital and Tumor Institute, Houston, Tex. These tumors were maintained in female DBA/2 mice (Simonsen Laboratories, Inc., Gilroy, Calif) by weekly transfer via IP inoculation of 10^6 cells as described elsewhere [2]. Testing for the antitumor activity of gossypol was done in BDF_1 mice following injection of 10^6 P388 cells or 10^5 L1210 cells. Gossypol at various concentrations was administered to mice IP at 24 h after their inoculation with tumor cells. Six mice were used for each treatment. The effect of gossypol on the life span of tumor-bearing mice was measured.

Histopathology of tissues. Mice were killed by cervical dislocation, and ascitic fluid was collected by flushing the peritoneal cavity with physiological saline. The cells were deposited on slides by means of a cytocentrifuge and stained with Giemsa for microscopic evaluation. Mice were dissected and the heart, lungs, liver, spleen, kidney, intestine, pancreas, and mesenteric lymph nodes were collected, fixed in phosphate-buffered 10% formaldehyde solution, embedded in paraffin, sectioned into 2- to 4-μm-thick slices, and stained with hematoxylin-eosin for microscopic observation. Bone marrow collected from the femur of each mouse was smeared on slides, fixed in methanol, stained with Wright's Giemsa, and scored for differential counts.

Drugs. High-purity gossypol was a generous gift from Dr Pemmaraju N. Rao of Southwest Research Foundation, San Antonio, Tex. We made a fresh preparation of gossypol by dissolving it in dimethylsulfoxide and then diluting it with physiological saline to give the desired concentration. The final concentration of dimethylsulfoxide in the saline was $\leq 0.2\%$ (v/v). Each mouse received 0.5 ml drug-containing saline as a single IP injection. In some experiments the drug was administered in two injections 1 or 2 days apart, depending upon the growth rate of tumor cells. Control animals received only saline.

Results

Toxicity of gossypol in BDF_1 mice

In the initial experiments, BDF_1 mice received various concentration of gossypol IP to allow a study of the toxic effects. Doses of 0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/mouse were tested. The effect of gossypol on survival of the mice was measured. No deaths were observed in mice that received 0.4 mg or less. In contrast, all mice that received 0.8 or 1.6 mg died (Table 1).

Antitumor effects of gossypol on Ca 755

On the basis of the toxicity data, we selected three different concentrations of gossypol, i.e., 0.4, 0.5, and 0.6 mg/mouse, to evaluate its antitumor effects. Control mice did not receive gossypol. Six mice were used for each treatment. The data presented in this paper represent a mean from two experiments. The animals were weighed at regular intervals to detect any increase in tumor size. There was no significant change in the body weight of control mice.

Table 1. Toxicity of gossypol in BDF_1 mice[a]

Dose	Average duration of survival (days)	Percent survival
Control (no drug)	>100	100%
0.05 mg/mouse	>100	100%
0.1 mg/mouse	>100	100%
0.2 mg/mouse	>100	100%
0.4 mg/mouse	>100	100%
0.8 mg/mouse	8	0
1.6 mg/mouse	5	0

[a] Gossypol dissolved in dimethylsulfoxide was diluted with saline and injected IP as a single dose in a volume of 0.5 ml. Control mice received only saline. Each treatment group consisted of four mice

In contrast, the tumor-bearing animals that received no gossypol exhibited a linear increase in body weight as a function of time, starting from day 6 after inoculation with 10^5 tumor cells per mouse (Fig. 1). By day 15, the abdomens of these mice were literally bloated with tumor cells. We never observed 'no takes' in the mice inoculated with tumor cells.

The drug-treated mice lost an average of 3–4 g in body weight during the first week after administration of the drug, but recovered fully afterwards. The higher the concentration of the drug, the longer was the delay before the body weight returned to the corresponding levels for control mice. At a dose of 0.6 mg/mouse the average body weight returned to normal levels and remained the same for >100 days, at which time the experiment was terminated. In mice that received 0.4 or 0.5 mg gossypol, not only was the lost body weight recovered but the weight also continued to increase with time (Fig. 1). The values in Fig. 1 represent the average for all the mice in each treatment group, consisting of both responders and nonrespon-

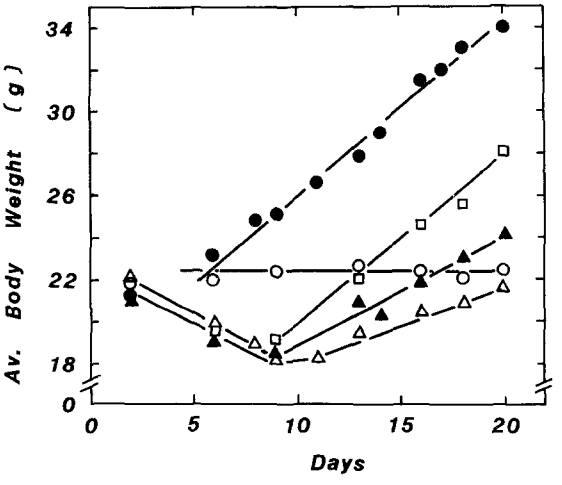

Fig. 1. Effect of gossypol on the body weight of Ca 755 tumor-bearing mice. Various doses of gossypol were administered 2 days after IP injection of 10^5 tumor cells per mouse. Body weight of each mouse was recorded at regular intervals and expressed as an average of six mice in each treatment
● – ●, control tumor-bearing mice that received no drug. Tumor-bearing mice received gossypol at doses of 0.4 mg/mouse, (□ – □), 0.5 mg/mouse (▲ – ▲), or 0.6 mg/mouse (△ – △). Controls (○ – ○) received neither tumor cells nor drug

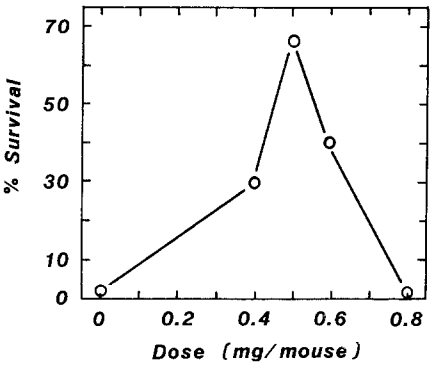

Fig. 2. Effect of gossypol on the long-term survival of Ca 755 tumor-bearing mice. These survivors were free of tumor cells and lived more than 100 days after injection of tumor cells, indicating complete cures by gossypol. The data point for the dose of 0.8 mg/mouse was taken from the data in Table 1

ders to drug treatment. If the values for the responders, in which tumor growth has been inhibited, were subtracted from the total, the average increase in body weight for nonresponders would be even more significant.

Ca 755 tumor exhibited a remarkable response to gossypol treatment. At an optimum dose of 0.5 mg/mouse, 66% of the animals were free of tumor and lived for more than 100 days, until the experiment was terminated (Fig. 2). The survival values for doses on either side of the optimum were significantly lower. The average duration of survival following inoculation of tumor cells in each treatment group would give a clue to the cause of death in these mice. In the controls, the tumor-bearing animals that received no gossypol died at about 18 days after inoculation of tumor cells (Table 2). This value is similar to that for the nonsurviving fraction of animals in groups that received 0.4 or 0.5 mg/mouse, suggesting that these mice died of tumor. In contrast, the nonsurviving fraction of animals in the group that received a higher dose of 0.6 mg/mouse died on average around day 10. This suggested that these animals were dying of drug toxicity rather than of tumor.

Effect of split dose of gossypol on the survival of Ca 755 tumor-bearing mice

We attempted to reduce the drug toxicity to mice without losing the antitumor effect by splitting the dose of gossypol and administering it on days 3 and 5. The group of mice that received a total of either 0.6 mg or 0.8 mg/mouse in two injections lived a couple of days longer than those that received the same amount of drug as a single injection (see Tables 1 and 2). Therefore, the split dose of gossypol appeared to be a little less toxic than a single dose but there was no antitumor activity. In contrast to the 40% and 66% long-term survivors among the mice that received gossypol in a single dose of 0.6 and 0.5 mg/mouse, respectively, there were no long-term survivors among the mice that received the drug in two instalments.

Effect of gossypol on cell number in the ascitic fluid

To determine the cause of death due to drug toxicity, control and tumor-bearing mice received 0.4, 0.5, or 0.6 mg gossypol per mouse injected as a single dose. Mice that received no gossypol served as controls. Mice were sacrificed at regular intervals up to 20 days after inoculation of tumor cells. At that time various tissues, including spleens, bone marrow, liver, kidney, heart, lungs, and messenteric lymph nodes, were subjected to histopathological examination. In addition, prior to the removal of tissue the peritoneal cavity was flushed with physiological saline and all the cells were collected and counted using a Coulter particle counter.

In tumor-bearing mice that received no gossypol the cell number in the peritoneal cavity increased exponentially until day 13, when it entered a plateau phase (Fig. 3). The peritoneal cell number was lowest in control animals, but it showed a sharp increase following IP injection of gossypol or tumor cells. In tumor-bearing mice that received 0.6 mg gossypol, the initial increase in the cell number in the peritoneal cavity gradually decreased by day 13 and remained low thereafter (Fig. 3). However, in the

Table 2. Effect of gossypol treatment on Ca 755 in BDF_1 female mice[a]

Dose	Average duration of survival following inoculation of 10^5 tumor cells on day 1 (days)[b]	% of animals survived for > 100 days
Control (no drug)	18.2	0
Single injection on day 3		
Gossypol 0.6 mg/mouse	10.6	40.0
Gossypol 0.5 mg/mouse	18.0	66.0
Gossypol 0.4 mg/mouse	20.6	30
Multiple injections		
Gossypol 0.3 mg/mouse on days 3 and 5 (Total dose 0.6 mg/mouse)	12.5	0
Gossypol 0.4 mg/mouse on days 3 and 5 (Total dose 0.8 mg/mouse)	10.2	0

[a] Gossypol was administered as a single IP injection on day 3, i.e., 2 days after the inoculation of tumor cells or split doses were given in two injections on days 3 and 5

[b] Mice that were cured of tumor and lived for > 100 days were not included in calculating the average duration of survival

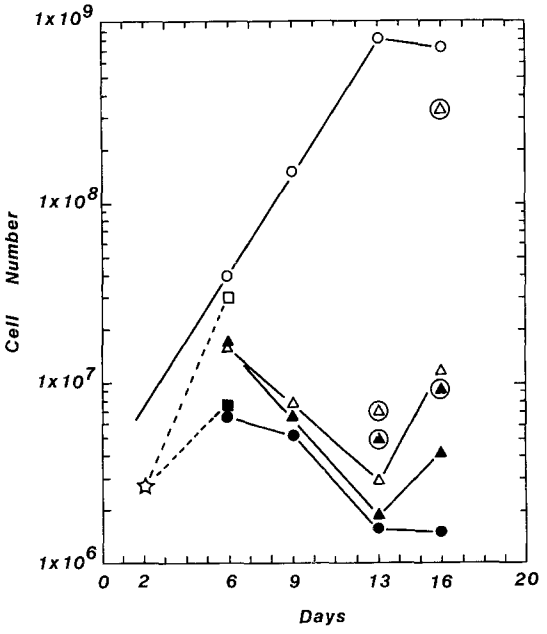

Fig. 3. Effect of gossypol on the number of cells in the peritoneal cavity of control and Ca 755 tumor-bearing mice. At regular intervals after inoculation of tumor cells and/or gossypol, three mice in each treatment group were sacrificed by cervical dislocation, their peritoneal cavity was flushed with a known volume of saline, and cells were counted using a Coulter counter. The data *points* represent averages of three mice. Some controls (*) received neither tumor cells nor drug, while others received no tumor cells but received gossypol at doses of 0.6 mg (■) or 0.5 mg (□) per mouse as single IP injections. Tumor-bearing mice receiving gossypol at doses of 0.6 (● – ●), 0.5 (▲ – ▲) or 0.4 mg/mouse (△ – △). ○ – ○ tumor-bearing mice that received no gossypol. ◬, ◭ individual mice that did not respond to gossypol at doses of 0.4 and 0.5 mg/mouse, respectively. The plotted data *points* were obtained by including these values. However, if these values for nonresponders are excluded, the average values for groups receiving 0.4 and 0.5 mg/mouse are similar to those receiving 0.6 mg/mouse

group of tumor-bearing mice that received 0.4 or 0.5 mg gossypol, the average (for 3 mice) cell number per peritoneal cavity started to increase after reaching a nadir on day 13. On days 13 and 16, one of the three mice in each group (receiving 0.4 or 0.5 mg gossypol) had a significantly higher number of cells in the peritoneal cavity than the rest (represented by open circles and solid triangles in Fig. 3). These mice represent nonresponders in which the drug had little or no cytotoxic effect. If the values for these animals were disregarded the average cell number per peritoneal cavity in these two groups would be similar to that of the group of mice that received 0.6 mg gossypol each. These data further confirm the results in Fig. 2: at low doses of gossypol the mice died of tumor, while at the high dose of 0.6 mg/mouse they died of drug toxicity.

Effect of gossypol on P388 and L1210 mouse leukemia

Each of 30 BDF_1 male mice received an IP injection of 10^6 P388 leukemia cells from a line maintained in DBA_2 mice. The 30 mice were divided into five groups of 6 each. At 24 h after inoculation with tumor cells mice received various concentrations of gossypol IP and were observed for the increase in life span of the treated mice. The average duration of survival after inoculation of tumor cells was 10.3 days in the untreated mice (Table 3). At higher concentrations of gossypol, i.e., 0.6 and 0.5 mg/mouse, the mice died much earlier, probably due to the combined effect of tumor and drug toxicity. However, at lower doses the duration of survival was similar to that of the untreated tumor-bearing mice (Table 3). These data clearly indicate that gossypol is not effective against this tumor when administerd as a single dose.

A similar study was conducted with L1210 leukemia using BDF_1 male mice following injections of 10^5 L1210 cells. On day 1 after the inoculation of tumor cells various concentrations of gossypol were administered IP. The increase in life span of the treated mice was recorded. Both the gossypol-treated and the untreated mice died around day 8, indicating the ineffectiveness of gossypol against this tumor (Table 4).

Administration of gossypol in two injections of equal amounts 24 h apart significantly reduced premature deaths among L1210-bearing mice due to drug toxicity, but did not have any antitumor activity (Table 4). These results are similar to those found with Ca 755 cells with regard to their response to split doses of gossypol.

Pathologic findings in gossypol-treated mice

The mean myeloid: erythroid (M: E) ratio in the bone marrow of healthy control mice was 1.4:1. The bone marrows were highly cellular (100%). Extensive extramedullary hematopoiesis and numerous megakaryocytes were observed, but they can be considered normal components of the red pulp in the mouse spleen.

The bone marrow of the tumor-bearing mice was not notably different from the controls. Compared with control mice, the tumor-bearing mice exhibited hyperplasia of lymphoid tissues and varying degrees of peritonitis. The mice given gossypol at doses of 0.6 and 0.5 mg had increased M: E ratios because of marked myeloid hyperplasia in some mice. The cellularity of the bone marrow was reduced. These mice also had varying degrees of peritonitis and lymphoid hyperplasia.

On day 12, the mice that did not receive gossypol had tumor cells in the tissues examined; mice treated with gossypol had no tumor cells. However, the untreated mice had myeloid hyperplasia, whereas most of those treated with gossypol had myeloid atrophy.

Table 3. Effect of gossypol on the survival of P388-bearing BDF_1 mice

Dose[a]	% Survival (long term)[b]	Average duration of survival (days)
A. Control (no drug)	0	10.3
B. Gossypol 0.6 mg/mouse	0	5.7
C. Gossypol 0.5 mg/mouse	0	5.7
D. Gossypol 0.4 mg/mouse	0	9.7
E. Gossypol 0.35 mg/mouse	0	10.8

[a] Gossypol was administered as a single IP injection on day 3, i.e., 2 days after the inoculation of tumor cells

[b] Long-term survival is defined as the survival of the animals for over 100 days following injection of tumor cells and gossypol

Table 4. Effect of gossypol on the survival of L1210-bearing BDF_1 mice

Dose[a]	% Survival (long term)[b]	Average duration of survival (days)
Control (no drug)	0	7.8
Single injection		
Gossypol 0.6 mg/mouse	0	3.7
Gossypol 0.5 mg/mouse	0	7.8
Gossypol 0.4 mg/mouse	0	8.2
Multiple injections		
Gossypol 0.4 mg/mouse on days 2 and 3 (Total dose 0.8 mg/mouse)	0	8.2
Gossypol 0.3 mg/mouse on days 2 and 3 (Total dose 0.6 mg/mouse)	0	8.0
Gossypol 0.3 mg/mouse on day 2 and 0.2 mg/mouse on day 3 (Total dose 0.5 mg/mouse)	0	8.75
Gossypol 0.25 mg/mouse given twice on days 2 and 3 (Total dose 0.5 mg/mouse)	0	8.75

[a] Gossypol treatment was given as a single IP injection on day 2, i.e., 24 h after the inoculation of tumor cells or split doses were given in two injections on days 2 and 3

[b] Long-term survival is defined as the survival of the animals for over 100 days following injection of tumor cells

Discussion

Earlier studies in our laboratory [18] showed that gossypol was a potent inhibitor of DNA synthesis but did not affect RNA and protein synthesis at the doses tested. In the presence of the drug, cells were blocked specifically in S phase. These observations suggested that gossypol had antitumor properties, and hence the present study was undertaken. The results of the present study proved this assumption to be correct at least with regard to the transplantable mouse mammary adenocarcinoma 755 in BDF_1 female mice. At an optimum dose of 0.5 mg/mouse given as a single injection 2 days after the inoculation of tumor cells, 66% of the tumor-bearing mice were rendered free of tumor cells, while the remaining 34% died of tumor. The percentage of long-term survivors could not be increased by increasing the dose of gossypol. Even a slight increase (0.1 mg/mouse) above the optimum dose resulted in the death of 60% of the treated mice due to drug toxicity. The effectiveness of gossypol decreased very rapidly below the optimum dose. Therefore, the effective dose range of gossypol is rather narrow. The antitumor effect of gossypol could not be attributed to peritonitis, since this condition was found in all tumor-bearing mice whether they received gossypol treatment or not.

When gossypol was administered in split doses to reduce drug toxicity to mice the antitumor effect was completely lost in the case of Ca 755 tumor (Table 2). These results suggest that drug toxicity is less than fully cumulative to the host, but the drug concentration does not reach the optimum level to exert antitumor effects. Even in the case of mice inoculated with L1210 tumor cells split doses of gossypol reduced the drug toxicity to the host but did not significantly increase the duration of survival. These findings are in agreement with our earlier report that there is a threshold concentration at which the drug becomes effective as a cytotoxic agent [18]. In the light of these observations it appears that multiple injections of gossypol in small doses would not be very effective in increasing the therapeutic index. However, in a recent study Tso [14] reported that IP injection of gossypol at an optimum daily dose of 100 μg/mouse over a period of 10 days into mice inoculated with Ehrlich ascites tumor cells resulted in a small (about 30%) but significant increase in the life span of these animals. However, a further increase in the dose of gossypol resulted in loss of body weight and the death of mice.

Earlier we reported that gossypol at a concentration of 10 μg/ml specifically inhibited DNA synthesis in cultured cells [18]. If the antitumor effects of gossypol in the Ca 755 tumor-bearing mice were entirely due to its inhibitory effects on DNA synthesis such an agent would not be expected to induce cures in 66% of the mice by a single injection. Usually S-phase-specific drugs are more effective when given as multiple injections over a period of time. However, in the present study the optimum dose was 500 μg per mouse with 20 g body weight, which is significantly higher than the 10 μg/ml used for in vitro studies. Furthermore, gossypol is known to exert a variety of effects, including potassium depletion in spermatozoa [14, 15], uncoupling of respiratory chain and oxidative phosphorylation [1, 7], and reduction of cellular ATP content [6, 16]. In view of the diversified effects of gossypol on cells it appears that the antitumor effects we observed in Ca 755 tumor-bearing mice may not be entirely due to its specific effects on DNA synthesis. Probably these Ca 755 tumor cells are more sensitive to the various effects of gossypol than either P388 or L1210 cells we have tested. Tso's [14] report indicates that Ehrich ascites tumor cells are responsive to gossypol to some extent. Therefore, it is possible that some tumors may be more sensitive to gossypol than others. Even though gossypol does not appear to be a promising antitumor agent, in view of its narrow therapeutic dose range, it is still worthwhile to identify the experi-

mental tumors that are sensitive to gossypol and then determine the cause for their sensitivity.

Histopathological studies of the tissues from gossypol-treated mice revealed no consistent lesions that could give an indication of organ-specific toxicity of gossypol. The reduced myeloid series in the gossypol-treated animals might have been due to depletion rahter than direct toxic effect on the bone marrow. Erythroid atrophy seen in some of these animals could have been a toxic effect or merely the anemia of "chronic infection", i.e., one associated with chronic peritonitis observed in tumor-bearing animals irrespective of gossypol treatment.

Acknowledgements. We thank Josephine Neicheril for her assistance in the preparation of this manuscript and G. Spotts for technical assistance. This study was supported in part by research grants CA 27544 und CA 34783 (to PNR) and CA 31394 (to EL) from the National Cancer Institute, DHHS.

References

1. Abdou-Dania MB, Dieckert JW (1974) Gossypol: Uncoupling of respiratory chain and oxidative phosphorylation. Life Sci 14: 1955
2. Avramis VI, Plunkett W (1983) Metabolism of 9-β-D-arabinosyl-2-flouroadenine-5'-phosphate by mice bearing P388 leukemia. Cancer Drug Delivery 1: 1
3. Bagg HJ, Jacksen J (1937) The value of a "functional test" in selecting material for a genetic study of mammary tumors in mice and rats. Am J Cancer 30: 539
4. Colman N, Gardner A, Herbert V (1979) Non-mutagenicity of gossypol in the Salmonella/mammalian-microsome plate assay. Environ Mutagen 1: 315
5. Kalle NR, Vasudev M (1981) Studies on the male antifertility agent-gossypol acetic acid. II: Effect of gossypol acetic acid on the motility and ATPase activity of human spermatozoa. Andrology 13: 95
6. Ke YB, Tso WW (1982) Variations of gossypol susceptibility in rat spermatozoa during spermatogenesis. Int J Fertil 27: 42
7. Killion DD, Shirley G, Frans RE (1968) Oxidative phosphorylative activities of mitochondria isolated from cotton hypocotyls. Plant Physiol 43: 1966
8. Laster WR Jr, Mayo JG, Simpson-Herren L, Griswold DP Jr, Lloyd HH (1969) Success and failure in the treatment of solid tumors. II: Kinetic parameters and "cell cure" of moderately advanced carcinoma 755. Cancer Chemother Rep 53: 169
9. Lotzova E, Savary, CA, Stringfellow DA (1983) Modulation of murine NK cell cytotoxicity in vitro and antitumor activity in vivo by low molecular weight interferon inducers. In Crispen RG (ed) Cancer: etiology and prevention. Elsevier Science, New York, p 199
10. Majumdar SK, Ingraham HJ, Prymowicz DA (1982) Gossypol an effective male contraceptive was not mutagenic in sperm head abnormality assay in mice. Can J Genet Cytol 24: 777
11. Montamat EE, Burgos C, de Burgos NM, Rovai LE, Blanco A, Segura EL (1982) Inhibitory action of gossypol on enzymes and growth of *Trypansoma cruzi*. Science 218: 288
12. National Coordinating Group of Male Antifertility Agents (1978) Gossypol – a new antifertility agent for males. Chin med J [Engl] 4: 417
13. Olgiati KL, Toscano WA (1983) Kinetics of gossypol inhibition of bovine lactate dehydrogenase X. Biochem. Biophys Res Commun 115: 180
14. Tso WW (1984) Gossyphol inhibits Ehrlich ascites tumor cell proliferation. Cancer Lett 24: 257
15. Qian S, Xu Y, Chen Z, Cao L, Sun S (1979) The influence of gossypol on the potassium metabolism of rats and the effect of some possible contributing factors. Acta Pharm Sinica 14: 513
16. Tso WW, Lee CS (1982) Potassium leakage: Not the cause of gossypol-induced antimobility in spermatozoa. Int J Androl 5: 317
17. Tso WW, Lee CS, Tso MYM (1982) The effect of gossypol on boar spermatozoal adenosine triphosphate metabolism. Arch Androl 9: 319
18. Tsui YC, Crasy MR, Hulten MA (1983) The effect of the male contraceptive agent gossypol on human lymphocytes in vitro: traditional chromosome-breakage, micronuclei, sister chromatid exchange, and cell kinetics. J Med Genet 20: 81
19. Wang YC, Rao PN (1984) Effect of gossypol on DNA synthesis and cell cycle progresion of mammalian cells in vitro. Cancer Res 44: 35
20. Ye WS, Liang JC, Hsu TC (1983) Toxicity of a male contraceptive, gossypol, in mammalian cell cultures. In Vitro 19: 53

Received October 29, 1984/Accepted January 2, 1985