Cancer Chemother Pharmacol (1992) 30: 480–482

Cancer Chemotherapy and Pharmacology

Short communication

A preliminary clinical study of gossypol in advanced human cancer

Robert C. Stein[1], A. E. A. Joseph[2], Stephen A. Matlin[3], David C. Cunningham[1], Hubert T. Ford[4], and R. Charles Coombes[1]

[1] Clinical Oncology Unit, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE, U. K.
[2] Department of Diagnostic Radiology, St. George's Hospital, Blackshaw Road, London SW17 OQT, U. K.
[3] Chemistry Department, The City University, Northampton Square, London EC1V OHB, U. K.
[4] Section of Radiotherapy, The Royal Marsden Hospital, Downs Road, Sutton, Surrey, U. K.

Received 14 September 1991/Accepted 20 March 1992

Summary. A total of 34 patients with advanced cancer were given weekly or daily escalating doses of oral gossypol, a cottonseed-oil constituent showing evidence of antineoplastic activity in pre-clinical studies. No major adverse events occurred and there was no evidence of haematological or biochemical disturbance. As determined by dose escalation in 17 patients, the dose-limiting toxicity was emesis in 16 patients. There was no evidence of tumour regression in any of the 20 patients assessed for response. We conclude that gossypol is safe but unlikely to be clinically useful in patients with advanced cancer.

Introduction

Gossypol, a phenolic compound (Fig. 1), occurs in racemic form in parts of the cotton plant and in raw cottonseed oil. The compound has undergone extensive clinical trials in China as a male antifertility agent [19]. Although the mechanism of action is unknown, gossypol disrupts a variety of cellular processes. These disruptions include inhibition of cytoplasmic and mitochondrial enzymes involved in energy production [9, 17] and uncoupling of oxidative phosphorylation [1, 15]; depletion of cellular adenosine triphosphate (ATP) has been demonstrated in cultured tumour cells [8]. Gossypol also inhibits key nuclear enzymes responsible for DNA replication and repair, including DNA polymerase alpha [13] and topoisomerase II [2], and blocks DNA synthesis in HeLa cells [18]. These properties make gossypol a potential antineoplastic agent. Gossypol is capable of inhibiting the growth of a variety of cell lines derived from epithelial tumours [3, 4, 16, 22] as well as that of several transplantable tumours [12, 14]. We therefore performed a phase I study of this drug in advanced human cancer.

Offprint requests to: R. C. Coombes

Patients and methods

A total of 34 patients with histologically proven advanced cancer that had ceased to respond to conventional systemic treatment or for which no effective systemic treatment was available were entered into a phase I study of oral gossypol. The median age of the patients enrolled in the study was 64 years (range, 40–82 years); 19 of the subjects were women. The median Karnofsky score of patients was 70; individuals with a score of less than 50 were excluded. Details of patients' primary tumour, disease sites and previous treatment are shown in Table 1. Patients gave informed consent and the study was approved by St. George's Hospital Ethics Committee.

Racemic gossypol acetic acid was purified to standard pharmacological levels of purity (Palmer Research Laboratories) and packaged in solid form into gelatin capsules in 30-mg doses. The study consisted of two parts. In the first part, 20 subjects in groups of 3–4 patients each received weekly escalating doses of gossypol ranging from 30 to 180 mg. In the second part, 14 subjects, again in groups of 3–4 patients each, were treated with repeat doses, which were given initially twice weekly, then daily and, finally, twice daily.

Patients were fully staged on entry by clinical examination, chest radiograph, measurement of complete blood count and serum biochemical parameters and, when clinically appropriate, by ultrasonography, computed tomographic (CT) scanning and bone radiology. Response to treatment was assessed according to International Union Against Cancer (UICC) criteria [11] at the end of the 6- to 10-week course of treatment. All treatments were carried out on an outpatient basis. During the initial stages of their treatment, patients were monitored weekly; the intervals between assessments were subsequently extended to 2 weeks. At each visit, patients were assessed for gossypol toxicity using a standard questionnaire. Complete blood counts, levels of serum urea and electrolytes, and liver-function tests were monitored weekly. Serum gossypol levels were measured in some patients at approximately 24 h after dosing; (+)

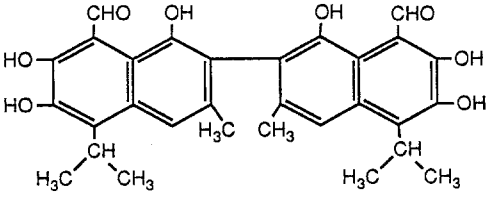

Fig. 1. Structure of gossypol

Table 1. Details of patients

Primary site	Number	Number previously treated with chemotherapy (number responding)	Sites of disease			
			Local	Visceral	Bone	Soft tissue
Oesophagus (squamous)	1	1 (0)	1	1	0	0
Upper GI tract	5	5 (2)	2	0	0	0
Cholangiocarcinoma	1	1 (1)	1	0	0	0
Colorectal	7	7 (1)	1	7	2	0
Lung (small-cell)	4	4 (3)	4	3	1	2
Lung (non-small-cell)	6	1 (0)	6	0	3	2
Breast	7	6 (1)[a]	1	3	2	7
Penis	1	1 (1)	0	1	0	1
Ovary	1	1 (1)	1	1	0	0
Myeloma	1	1 (1)	1	0	1	0
Totals	34	28 (11)				

[a] All 7 breast-cancer patients had also previously received endocrine therapy, to which 2 responded

and (–)-enantiomers were determined by derivatisation with phenylalanine methylester prior to extraction and reverse-phase high-performance liquid chromatography (HPLC) according to published methods [10].

Results

In all, 20 patients were treated once weekly with gossypol at doses ranging from 30 to 180 mg. The duration of treatment varied from 1 to 21 (median, 4) weeks. Subsequently, 14 patients were treated with escalating daily doses of gossypol; the initial dose was progressively increased from 30 mg twice weekly to 30 mg twice daily. Toxicity data were available for 28 patients; the remaining 5 subjects were lost to follow-up.

Serum gossypol levels were measured at approximately 24 h after dosing in 7 patients receiving weekly treatment. Gossypol was detectable in the serum (>50 mg/ml) of 6 patients, with levels varying between 77 and 625 mg/ml. The ratio of (–)- to (+)-enantiomers varied both between patients and within individuals in whom more than one measurement was made, but it tended to be less than 1 (median, 0.8). In this small group of patients, no clear correlation was found between serum drug levels and the gossypol dose.

Toxicity

The maximum tolerable dose of gossypol was determined in 17 patients by dose escalation. The median limiting dose for patients receiving daily treatment was 30 mg, and that for patients receiving weekly gossypol was 120 mg. Toxic side effects encountered included emesis (n = 16), diarrhoea (n = 3) and lethargy (n = 1). Emesis was recorded as being severe in 13/16 patients and was only partly controlled by the administration of domperidone and prochlorperazine. However, despite the use of anti-emetics, most of these patients felt unable to continue the study. Liver metastases did not appear to predispose patients to gossypol-induced emesis. No disturbance in haematological or biochemical parameters other than those related to the underlying disease was noted during the study. In particular, there was no evidence of drug-related bone marrow toxicity or hypokalaemia.

Therapeutic effect

Overall, 23 patients completed 3 or more weeks of treatment, of whom 13 received gossypol daily. In all, 20 patients were assessable for response, 2 were lost to follow-up and 1 was withdrawn because of intolerance. No patient showed any evidence of tumour regression during treatment. In 2 patients with squamous-cell lung carcinoma and 1 with metastatic breast cancer, the disease remained stable for 16, 23, and 19 weeks, respectively. All other assessable patients developed progressive disease during the treatment period.

Discussion

In this preliminary clinical study, we found that oral gossypol can safely be given to patients with advanced cancer. Emesis, the main subjective toxicity, appeared to be dose-related. The doses we used are comparable with the 20-mg daily dose that was widely given in the Chinese contraceptive studies [19]. The lack of myelotoxicity in our patients is consistent with the findings of previous animal studies and clinical trials [19] and with the observation that cultured human bone marrow is relatively more resistant to the growth-inhibitory actions of gossypol than cultured tumour cell lines [4]. The complete lack of clinical activity in the present study suggests that gossypol is unlikely to prove to be of great utility in the treatment of cancer. However, due to the adverse clinical features of the patient population investigated in our study, we cannot exclude clinical activity in patients with more favourable disease. Gossypol has recently been reported to cause tumour regression in a case of advanced adrenocortical cancer [5].

We used the racemic form of gossypol in our study. Gossypol is known to be heavily protein-bound, especially the (+)-enantiomer, and previous systematic pharmacokinetics studies have shown that this binding results in significantly slower excretion of the (+)-enantiomer [20, 21]. Although we did not perform systematic pharmacokinetics studies since these have previously been carried out [20], we detected a preponderance of the (+)-enantiomer in serum samples obtained from our patients, which is consistent with the aforementioned observation.

The levels of gossypol that we found in serum samples were generally rather lower than those that have been used in growth-inhibitory studies in tissue-culture models. Furthermore, the role of protein binding in reducing the proportion of free gossypol to total drug is likely to be greater in human serum, which contains higher total protein levels than do tissue-culture media. It has previously been noted that the cytotoxicity of gossypol may be reduced by increasing the serum content of tissue-culture media [6, 7]. It may therefore be of considerable therapeutic significance that the less tightly protein-bound (–)-enantiomer is more cytotoxic in vitro [3, 7]. We suggest that any further clinical trials of gossypol should use the (–)-enantiomer, whose therapeutic index may well be substantially greater than that of the racemic mixture.

References

1. Abou-Donia MB, Dieckert JW (1974) Gossypol: uncoupling of respiratory chain and oxidative phosphorylation. Life Sci 14: 1955
2. Adlakha RC, Aschorn CL, Chan D, Zwelling LA (1989) Modulation of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide-induced, topoisomerase II-mediated DNA cleavage by gossypol. Cancer Res 49: 2052
3. Band V, Hoffer AP, Band H, Rhinehardt AE, Knapp RC, Matlin SA, Anderson DJ (1989) Antiproliferative effect of gossypol and its optical isomers on human reproductive cancer cell lines. Gynecol Oncol 32: 273
4. Benz C, Keniry M, Goldberg H (1988) Selective toxicity of gossypol against epithelial tumors and its detection by magnetic resonance spectroscopy. Contraception 37: 221
5. Flack MR, Wu YW, Reidenburg MA, Pyle RG, Mullens NM, Nisula BC, Loriaux DL (1990) Treatment of adrenocortical carcinoma with gossypol. Proc Am Assoc Cancer Res 31: 198
6. Haspel H, Ren YF, Watanabe KA, Sonenberg M, Corin RE (1984) Cytocidal effect of gossypol on cultured murine erythroleukemia cells is prevented by serum protein. J Pharmacol Exp Ther 229: 218
7. Joseph AEA, Matlin SA, Knox P (1986) Cytotoxicity of enantiomers of gossypol. Br J Cancer 54: 511
8. Keniry MA, Hollander C, Benz CC (1989) The effect of gossypol and 6-aminonicotinamide on tumor cell metabolism: a ^{31}P-magnetic resonance spectroscopic study. Biochem Biophys Res Commun 164: 947
9. Lee CYG, Moon YS, Yuan JH, Chen AF (1982) Enzyme inactivation and inhibition by gossypol. Mol Cell Biochem 47: 65
10. Matlin SA, Belenguer A, Vince PA, Stein RC (1990) Analysis of gossypol enantiomers in human serum. J Liquid Chromatogr 13: 2261
11. Miller AB, Hoogstraten B, Straquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207
12. Rao PN, Wang YC, Lotzova E, Khan AA, Rao SP, Stephens LC (1985) Antitumor effects of gossypol on murine tumors. Cancer Chemother Pharmacol 15: 20
13. Rosenberg LJ, Adlakha RC, Desai DM, Rao PN (1986) Inhibition of DNA polymerase alpha by gossypol. Biochim Biophys Acta 866: 258
14. Tso WW (1984) Gossypol inhibits Ehrlich ascites tumor cell proliferation. Cancer Lett 24: 257
15. Tso WW, Lee CS (1982) Gossypol uncoupling of respiratory chain and oxidative phosphorylation in ejaculated boar spermatozoa. Contraception 25: 649
16. Tuszynski GP, Cossu G (1984) Differential cytotoxic effect of gossypol on human melanoma, colon carcinoma, and other tissue culture cell lines. Cancer Res 44: 768
17. Ueno H, Sahni MK, Segal SJ, Koide SS (1988) Interaction of gossypol with sperm macromolecules and enzymes. Contraception 37: 333
18. Wang YC, Rao PN (1984) Effect of gossypol on DNA synthesis and cell cycle progression of mammalian cells in vitro. Cancer Res 44: 35
19. Wu DF (1989) An overview of the clinical pharmacology and therapeutic potential of gossypol as a male contraceptive agent and in gynaecological disease. Drugs 38: 333
20. Wu DF, Yu YW, Tang ZM, Wang MZ (1986) Pharmacokinetics of (+/–), (+), (–) gossypol in humans and dogs. Clin Pharmacol Ther 39: 613
21. Wu DF, Reidenberg MM, Drayer DE (1988) Determination of gossypol enantiomers in plasma after administration of racemate using high-performance liquid chromatography with precolumn chemical derivatisation. J Chromatogr 433: 141
22. Wu YW, Chik CL, Knazek RA (1989) An in vitro and in vivo study of antitumour effects of gossypol on human SW-13 adrenocortical carcinoma. Cancer Res 49: 3754