In vitro and in vivo cytotoxicity of gossypol against central nervous system tumor cell lines

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Summary

Gossypol is a lipid soluble polyphenolic compound isolated from cotton seed oil which has been previously shown to have antiproliferative activity in vitro against a variety of human solid tumor cell lines. It has been extensively tested in clinical trials as a male contraceptive agent and found to be well tolerated. Its mechanism of action is thought to be inhibition of cellular energy metabolism. It inhibits glycolysis through inhibition of LDH isoenzyme type 5, and it inhibits mitochondrial oxidative phosphorylation and electron transport. We tested the in vitro antiproliferative effect of gossypol against four well characterized human glioma cell lines, HS 683, U373, U87 and U138, and one rat glioma cell line, C6, using the colorimetric Microculture Tetrazolium Assay (MTT). Cytotoxicity was found to be concentration and time dependent and increased with incubation times up to 8 days. The relative sensitivity of the glioma cell lines to gossypol at 48 hour incubation correlated with their respective LDH isoenzyme profiles, with the more sensitive cell lines expressing increased cathodal LDH isoenzymes (LDH 5). The in vitro cytotoxicity of gossypol to these CNS tumor lines was compared to the other non central nervous system solid tumor cell lines which had been previously reported as being sensitive to gossypol, including SW-13 (adrenal), MCF-7 (breast), T47-D (breast), and HeLa (cervical). Additional lines tested included SK-MEL-3 (melanoma), Colo 201 (colon) and BRW, a line established in our laboratory from a patient with a Primitive Neuroectodermal tumor. C6, HS 683, and BRW had similar IC50s as the sensitive solid tumor cell lines. U373, U87 and U138 had significantly less sensitivity at 48 hours. There was greater cytotoxicity and no significant differences in the IC50s between any of cell lines at 8 day incubations.

Additionally, we tested the cytotoxicity of gossypol against BRW in vivo, using the nude mouse xenograft model. Gossypol, given at a dose of 30 mg/kg per day five days a week for four weeks orally via gavage, was found to decrease the mean tumor weight of treated xenografts by more than 50% as compared to untreated xenografts. These findings suggest that gossypol has potential for further study as an agent for the treatment of primary CNS malignancies.

Introduction

Gliomas occur at a rate of approximately 5 per 100,000 per year and the incidence may be increasing [1]. Over half are high grade gliomas and these patients have an extremely poor prognosis. In a recent retrospective analysis of 285 patients with high grade glioma treated with cytoreductive surgery and radiotherapy, the median survival for all patients was only 35 weeks [2]. The prognosis for elderly patients with high grade glioma is even worse, with a 16 week median survival reported for patients over 60 treated with cytoreductive surgery and radiotherapy [3]. Currently available chemotherapy is estimated to decrease the tumor cell burden by only one log, which is two logs less than the
decrease estimated to be necessary for curative potential when given in conjunction with radiotherapy and surgery [4]. It is generally accepted that nitrosoureas achieve response rates of approximately 50% in phase II studies in recurrent glioma [5]. Procarbazine used as a single agent has been found to have a response rate of approximately 27% using modern response criteria [6]. Combination chemotherapy with regimens such as Procarbazine, CCNU and Vincristine (PCV) may have higher response rates than single agent therapy with BCNU, but the advantage is slight [7]. A recent meta-analysis of adjuvant trials of alkylating agent based chemotherapy in high grade glioma revealed an overall modest survival benefit and increased time to progression for chemotherapy treated patients as opposed to controls. However, the benefit was not demonstrable in all subgroups and was limited to patient groups who had anaplastic astrocytoma [8]. Efforts to increase the efficacy of standard chemotherapy by escalating doses with autologous bone marrow rescue may improve response rates somewhat, but the applicability of this approach is somewhat limited by the inability of elderly patients to withstand these rigorous and expensive treatments. Clearly other strategies must be explored in the treatment of high grade glioma.

The development of new drugs with anti-glioma activity remains an important strategy. New agents which are relatively non-toxic and better tolerated by older patients, who make up the majority of the patients with high grade glioma, will be especially useful. Rosenblum et al. hypothesized that ‘chemotherapy programs that are designed on the basis of metabolic capabilities of the target cells should be more successful than those that are not’ [9]. The well described differences in the energy metabolism between normal brain and glioma cells are attractive targets for exploiting this strategy. These differences [10] are summarized as follows: Within the tumor cells there is: 1) A surplus of ATP; 2) A striking decrease in oxidative phosphorylation and activity of the Krebs cycle; 3) A striking increase in glycolysis even in the presence of oxygen (aerobic glycolysis), which allows for energy production in the tumor even under hypoxic conditions as long as an adequate supply of glucose is available; 4) An increase in glucose utilization; 5) An increase in the activity of the pentose phosphate shunt; 6) And a striking decrease in the number of mitochondria. While total LDH activity has been shown to be normal in glioma tissue, the isoenzyme pattern of glioma cells is shifted to the cathodal form of LDH: LDH5. This shift is correlated to the degree of anaplasia, with a shift being seen in 79% of grade III/IV tumors but only 14% of grade I/II tumors [11]. The cathodal form of LDH is generally associated with anaerobic glycolysis and is found normally in muscle tissue.

Gossypol, a polyphenolic, lipid soluble compound, isolated from cotton seed oil is a drug which could potentially exploit the differences in energy metabolism. Its structure is shown in Fig. 1. Gossypol has been shown to have antiproliferative activity in vitro against a number of tumor cell lines including melanoma [12], colon [12], cervical [13], adrenocortical [14] and breast [15]. It has been shown that gossypol uncouples electron transport, and inhibits oxidative phosphorylation, ATP production [16], and glycolysis [17]. Gossypol’s inhibition of glycolysis is thought to be mediated by inhibition of Lactate dehydrogenase. Of the LDH isoenzymes, gossypol specifically inhibits LDH-5 and LDH-X (the form of LDH found in sperm) [18]. Studies by Benz et al. [13], and Tuszynski et al. [12], have shown that the in vitro antiproliferative effect of gossypol is correlated to the LDH profile of various cell lines tested, with lines shifted to LDH-5 being more sensitive.

Gossypol has been tested extensively in humans in clinical trials as a male contraceptive agent [19], as well in two clinical trials as an antineoplastic agent [20, 21], and has been found to be well tolerated. We undertook this study to determine the in vitro sensitivity of glioma cell lines to gossypol, and to