Pharmacokinetics and antitumor properties in tumor-bearing mice of an enediol analogue inhibitor of glyoxalase I

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Abstract Purpose: The enediol analogue S-(N-p-chlorophenyl-N-hydroxycarbamoyl)glutathione (CHG) is a powerful, mechanism-based, competitive inhibitor of the methylglyoxal-detoxifying enzyme glyoxalase I. The [glycylglutamyl]diethyl ester prodrug form of this compound (CHG(Et)2) inhibits the growth of different tumor cell lines in vitro, apparently by inducing elevated levels of intracellular methylglyoxal. The purpose of this study was to evaluate the pharmacokinetic properties of CHG(Et)2 in plasma esterase-deficient C57BL/6 (Es-1) mice after intravenous (i.v.) or intraperitoneal (i.p.) administration of bolus doses of CHG(Et)2. In addition, the in vivo antitumor properties of CHG(Et)2 were evaluated against murine B16 melanoma in these mice, and against androgen-independent human prostate PC3 tumor and human colon HT-29 adenocarcinoma in plasma esterase-deficient nude mice. Methods: Pharmacokinetics were evaluated after either i.v. or i.p. administration of CHG(Et)2 at the maximally tolerated dose of 120 mg/kg to both tumor-free male and female mice and male and female mice bearing subcutaneous B16 tumors. Tissue concentrations of CHG(Et)2, CHG and the [glycyl]monoethanol ester CHG(Et) were measured as a function of time by reverse-phase C18 high-performance liquid chromatography of deproteinized tissue samples. The efficacy of CHG(Et)2 in tumor-bearing mice was evaluated after i.v. bolus administration of CHG(Et)2 at 80 or 120 mg/kg for 5 days each week for 2 weeks, or after 14 days continuous infusion of CHG(Et)2 using Alzet mini-osmotic pumps. Hydroxpropyl-β-cyclodextrin was used as a vehicle in the efficacy studies. Results: Intravenous administration of CHG(Et)2 resulted in the rapid appearance of CHG(Et)2 in the plasma of tumor-bearing mice with a peak value of 40–60 μM, followed by a first-order decrease with a half-life of about 10 min. There was a corresponding increase in the concentration of inhibitory CHG in the B16 tumors, with a maximum concentration in the range 30–60 μM occurring at 15 min, followed by a decrease to a plateau value of about 6 μM after 120 min. Neither CHG(Et)2 nor its hydrolysis products were detectable in plasma, after i.p. administration of CHG(Et)2 to tumor-free female mice. From the efficacy studies, dosing schedules were identified that resulted in antitumor effects comparable to those observed with the standard antitumor agents Adriamycin (with B16 tumors), cisplatin (with PC3 tumors), and vincristine (with HT-29 tumors). Conclusion: This is the first demonstration that a mechanism-based competitive inhibitor of glyoxalase I effectively inhibits the growth of solid tumors in mice when delivered as the diethyl ester prodrug.

Key words S-(N-p-chlorophenyl-N-hydroxycarbamoyl) glutathione · Glyoxalase I inhibitor · Pharmacokinetics · In vivo efficacy

Introduction

Competitive inhibitors of glyoxalase I have long been proposed as possible anticancer agents because of their potential ability to induce elevated levels of cytotoxic methylglyoxal in tumor cells [22]. In 1992, Lo and Thornalley obtained experimental support for this hypothesis with the demonstration that the glyoxalase I inhibitor, S-p-bromobenzylglutathione, is efficiently delivered into human leukemia HL60 cells as the diethyl ester prodrug and inhibits cell growth [11]. Work carried out in the same laboratory subsequently showed that the
dicyclopentyl ester prodrug is cytostatic to several different human tumors in vitro [20], and a murine adenocarcinoma in vivo [19]. Glyoxalase I inhibitors are potentially selective tumoricidal agents, as extracellular methylglyoxal is known to inhibit preferentially the growth of rapidly dividing tumor cells as compared to quiescent normal cells in tissue culture [2, 4, 14, 15]. Selective toxicity might be due to inhibition of DNA synthesis [15, 24]. Indeed, methylglyoxal is known to form covalent adducts with both proteins and nucleic acids [13].

We recently evaluated the in vitro antitumor properties of three mechanism-based competitive inhibitors of glyoxalase I as the diethyl ester prodrugs, e.g. the [glycylglutamyl]diethyl ester prodrug form of S-(N-p-chlorophenyl-N-hydroxycarbamoyl)glutathione (CHG) (CHG(Et)2; Fig. 1) [10]. CHG is one of the strongest competitive inhibitors of human glyoxalase I yet reported (Kx. 40 nM). CHG(Et)2 inhibits the growth of L1210 murine leukemia (G150 7 μM) and the solid murine tumor B16 melanotic melanoma (G150 15 μM) in vitro. Growth inhibition is accompanied by rapid esterase-catalyzed conversion of CHG(Et)2 to the inhibitory diacid CHG inside the cells. That growth inhibition reflects inhibition of glyoxalase I is consistent with the observation that preincubation of L1210 cells with CHG(Et)2 increases the sensitivity of the cells to inhibition by extracellular methylglyoxal [10]. Moreover, L1210 cells are more sensitive than murine splenic lymphocytes to the inhibitory effects of CHG(Et)2. This could reflect the greater intrinsic sensitivity of rapidly dividing cells to methylglyoxal. Also, selectivity could arise from the ten-fold lower activity of glyoxalase II in L1210 cells as compared to splenic lymphocytes [10]. Because CHG is slowly hydrolyzed by GlxII, CHG might be more stable in L1210 cells and, therefore, more toxic to these cells. This mechanism of selective toxicity might apply generally, as many different types of cultured and excised tumors contain abnormally low levels of glyoxalase II activity, including the three tumor types examined in the current study [3, 6, 8].

We describe here the pharmacokinetics and antitumor properties of CHG(Et)2 in plasma esterase-deficient C57BL/6 (Es-1E) mice bearing murine B16 melanoma, and in esterase-deficient athymic nude mice bearing androgen-independent human prostate PC3 or human colon HT-29 xenografts. The importance of the plasma esterase-deficient mice used in this study is that they model the low esterase activity in human plasma [10]. This is the first systematic preclinical evaluation of the chemotherapeutic potential of a mechanism-based competitive inhibitor of glyoxalase I.

### Materials and methods

#### Reagents

The glyoxalase I inhibitor CHG was synthesized via an acyl interchange reaction between glutathione and N-p-chlorophenyl-N-hydroxycarbamate p-chlorophenyl ester, as previously described by this laboratory [12]. This procedure was scaled up to produce gram quantities of CHG as follows. A solution of freshly synthesized and crystallized N-p-chlorophenyl-N-hydroxycarbamate p-chlorophenyl ester (4.6 g, 15.5 mmol) was prepared in 350 ml absolute ethanol. To this solution was added over a period of approximately 30 min with stirring a freshly prepared solution of glutathione (29 g, 84.6 mmol) in degassed nitrogen-saturated water at pH 9.5. The reaction mixture was placed under an atmosphere of nitrogen and stirred at room temperature until little acetylating agent remained (about 24 h) as judged by silica gel TLC (n-propanol/acetic acid/water, 10:1:5 v/v/v). The acetylating reagent and the product had Rf values of 0.89 (UV) and 0.61 (UV, ninhydrin), respectively. The reaction mixture was adjusted to a pH meter reading of 3.5 with 6 N HCl and the solvent removed in vacuo. The white residue was suspended in 200 ml water and stirred at room temperature overnight. The solid was removed by filtration, suspended in 250 ml water and stirred for about 6 h. The white precipitate was again collected by filtration and thoroughly dried under vacuum. In order to remove unreacted acetylating reagent and p-chlorophenol, the precipitate was suspended in 300 ml diethyl ether and stirred overnight. This washing procedure was repeated two more times (stir times of about 3 h) in order to remove the last traces of acetylating reagent and p-chlorophenol as judged by silica gel TLC (yield 60%; analytical data identical to literature values [12]). The corresponding diethyl ester CHG(Et)2 was prepared by incubation of CHG in 6.2 N ethanolic HCl at room temperature until diesterification was >95% complete (about 8 h). The progress of the reaction was monitored by reverse phase C18 column chromatography (Waters µBondapak C18, 7.8 x 300 mm), using 0.25% acetic acid and 40% methanol in water as an eluting solvent (2 ml/min). The approximate retention times were as follows: CHG(Et)2, 76 min, CHG 27 min, CHG[glycyl][Et] 62 min. The dicarbonyl diester CHG(cyclopentyl)2 was similarly prepared by incubating CHG in cyclopentanol/HCl.

The metabolites S-(N-p-chlorophenyl-N-hydroxycarbamoyl) cysteinylglycine and S-(N-p-chlorophenyl-N-hydroxycarbamoyl) cysteine were prepared by partial hydrolysis of CHG using the peptidases present in mouse kidney homogenate. To 5 ml of a