Changes in glutathione-related enzymes in tumor-bearing mice after cisplatin treatment

D. Khynriam and S. B. Prasad
Cell and Tumor Biology Laboratory, Department of Zoology, North-Eastern Hill University, Shillong, India

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Abstract

The effect of cisplatin on five glutathione-related enzymes was studied in liver, kidney, and Dalton lymphoma cells of tumor-bearing mice. In liver, the activities of glutathione S-transferase, glutathione peroxidase, catalase, and superoxide dismutase decreased approximately 30–40%, 60–67%, 35–50% and 70–80% respectively, while glutathione reductase increased about 36–45% after cisplatin treatment. In kidney, catalase activity decreased by 47–82% at all time points (24–96 h) of cisplatin treatment, while glutathione S-transferase activity decreased significantly (~24%) mainly at 72 h of treatment. An increase in glutathione reductase (~1.5–2.5 times), glutathione peroxidase (significant at 24 h, 47%), and superoxide dismutase (~15–60%) was noted in kidney after the treatment. In Dalton lymphoma cells, the activities of glutathione S-transferase, glutathione peroxidase, and catalase decreased very distinctly (~2–5, 2–5 and 5–11 times, respectively) at all time points, but glutathione reductase decreased significantly only at 72 h of cisplatin treatment. Interestingly, the superoxide dismutase activity in Dalton lymphoma cells increased initially at 24–48 h and then decreased (~60%) during later periods (72–96 h) of treatment. Cisplatin treatment caused a decrease in glutathione level in Dalton lymphoma cells (~14–20%) and kidney (~18–28%) but no change in liver. In view of the results, a definite correlation with the changes in glutathione concentrations and enzymatic activities in a tissue could not be firmly derived. It is suggested that the changes in various glutathione-related enzymes and glutathione levels in the tissues of the host during cisplatin-mediated chemotherapy could affect cellular antioxidant defense potential, which may play an important contributory role in cisplatin-mediated toxicity, particularly nephrotoxicity, and anticancer activity in the host.

Abbreviations: DL, Dalton lymphoma; GSH, reduced glutathione; GST, glutathione S-transferase; GR, glutathione reductase; GPx, glutathione peroxidase; CAT, catalase; SOD, superoxide dismutase; GSSG, oxidized glutathione or glutathione disulfide; CDNB, 1-chloro-2,4-dinitrobenzene; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; NADPH, nicotinamide–adenine dinucleotide phosphate, reduced
Introduction

cis-Diaminedichloroplatinum(II), commonly known as cisplatin, is established to be an effective chemotherapeutic drug against various malignancies (Prasad and Giri, 1994; Go and Adjei, 1999). Besides its ability to interact with cellular DNA (Zwelling et al., 1979; Coste et al., 1999), its effects on the host immune response (Collins and Kao, 1989), cell surface (Prasad and Sodhi, 1981), tissue calcium and potassium concentrations (Prasad and Giri, 1999), various enzymes such as S'-nucleotidase, arginase, cathepsins and lactate dehydrogenase (Prasad et al., 1999), and mitochondria (Kharbangar et al., 2000) have also been observed, and it has been suggested that these changes are also involved as additional components in the mechanism of cisplatin’s anticancer activity. However, full clinical therapeutic efficacy of cisplatin is limited by its major side-effects of nephrotoxicity (Krakoff, 1979) and also hematotoxicity (Khnriam and Prasad, 2001).

Glutathione (a tripeptide: L-γ-glutamyl-L-cysteinylglycine), usually the most prevalent intracellular thiol, functions directly or indirectly in a variety of cellular processes (Wang and Ballatori, 1998). Reduced glutathione (GSH) plays an important role in defense mechanisms by acting as an antioxidant or by reacting with electrophiles (Delevé and Kaplowitz, 1991) and toxic agents to form conjugates that are eliminated from the cell (Meister, 1991). Elevation of GSH in cellular resistance to platinum agents has been reported in several human and murine tumor cell lines (Kartalou and Essigmann, 2001) and the enzymes of glutathione metabolism play a role in cellular resistance to anticancer drugs (Black and Wolf, 1991). GSH participates in the detoxification of xenobiotics that cause cellular injury by generating free radicals (Slater, 1984; Sies, 1999). Intracellular defense mechanisms to detoxify the reactive free radicals include the GSH-related enzymes, viz., glutathione S-transferase (EC 2.5.1.18), glutathione reductase (EC 1.6.4.2), glutathione peroxidase (EC 1.11.1.9), superoxide dismutase (EC 1.15.1.1), and catalase (EC 1.11.1.6) (Tew, 1994; Ohkuwa et al., 1997; Teramoto et al., 1999). We have reported that cisplatin-mediated decrease in GSH concentrations could play an important role in the development of mutagenicity (Giri et al., 1998a), nephrotoxicity (Giri et al., 1998b), and hematotoxicity (Khnriam and Prasad, 2001) in the host and proposed that the GSH-related enzymes may be affected in the mechanism of cisplatin-mediated toxicity and anticancer activity (Khnriam and Prasad, 2001).

The details of the changes and significance of GSH-related enzymes in cisplatin-mediated anticancer activity or toxicity and changes in GSH concentrations is not clearly understood. As cisplatin is known to develop major side-effects of nephrotoxicity, kidneys were used along with liver (the major site of GSH metabolism) and tumor cells for the study. The present investigations were undertaken to elucidate the changes in the activity of some GSH-related enzymes in liver, kidney, and Dalton lymphoma (DL) cells of tumor-bearing mice treated with cisplatin for different periods of time.

Materials and methods

Chemicals

Reduced glutathione, oxidized glutathione (GSSG), glutathione reductase, and 5,5'-dithiobis-2-nitrobenzoic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. Cisplatin was obtained from Biochem Pharmaceutical Industries, Mumbai, India. 1-Chloro-2,4-dinitrobenzene (CDNB), nicotinamide–adenine dinucleotide phosphate reduced (NADPH), hydrogen peroxide, sodium azide,