

Methionine Starvation Modulates the Efficacy of Cisplatin on Human Breast Cancer in Nude Mice

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Abstract. *There are few agents with activity against metastatic breast cancer. We therefore exploited the elevated methionine dependence of tumors to develop a selective and effective therapy against metastatic breast and other cancers. Methionine starvation leads to depleted methionine levels in cells, modifies methylation reactions, lowers glutathione levels and alters folate distribution and leads to a tumor-selective cell cycle arrest in late-S/G₂. These effects present the opportunity for methionine depletion to modulate the efficacy of a number of different classes of chemotherapeutic drugs. This report demonstrates that methionine depletion can strongly modulate the efficacy of cisplatin against the MX-1 human breast carcinoma cell line when grown in nude mice. The tumor-bearing nude mice were subjected to a methionine-free diet and were additionally treated with cisplatin i.p. at one mg/kg once a week for 3 weeks. The MX-1 tumor was relatively resistant to both methionine starvation and cisplatin alone but was very sensitive to the combination of methionine starvation and cisplatin with a 32.1% T/C ratio. The intratumoral platinum concentration was higher in combination with methionine starvation than cisplatin alone, possibly accounting for at least part of the modulating effect of methionine depletion. Future studies will focus on methionine depletion via the enzyme methioninase to modulate cisplatin as well as other classes of chemotherapeutic agents in order to develop a new approach to the treatment of cancer.*

Methionine (MET)-dependence denotes the inability of tumor cells to grow under the conditions MET-depletion. MET-dependence has been observed in many types of human malignant tumors *in vitro* (1, 2) and *in vivo* (3), but not in normal cells or tissues.

Under conditions of MET-depletion, MET-dependent cancer cells, unlike normal cells, arrest in the late-S/G₂ phase of the cell cycle. The tumor-selective late -S/G₂ arrest due to MET-depletion has been observed *in vitro* (4), and *in vivo* (5). This tumor-specific cell cycle arrest was exploited in combination with chemotherapeutic agents *in vitro* to selectively eliminate tumor cells from cocultures with normal cells (6).

However, no studies have been done as yet to determine how MET-depletion can modulate the efficacy of chemotherapeutic agents on a human tumor *in vivo*. Cisplatin is widely used in combination chemotherapy of solid tumors. However, complete responses are rarely observed in these solid tumors. Therefore, more effective combination chemotherapy regimens containing cisplatin are necessary. This is the first report indicating that MET-depletion could be a modulator of cisplatin efficacy against a human tumor xenograft *in vivo*.

Materials and Methods

Female BALB/c *nu/nu* mice were obtained from the Central Institute for Experimental Animals (Kawasaki, Japan). Mice, 6-8 weeks old and weighing 20-22 g were used. The human breast cancer xenograft, MX-1 was used in these studies (7). Tumors in the exponential growth phase in nude mice were resected aseptically, necrotic tissues were cut away, and the remaining viable tumor tissue was cut into blocks approximately 3 to 4 mm in diameter in Hanks' balanced salt solution. One block was transplanted subcutaneously on the backs of nude mice under ether anesthesia.

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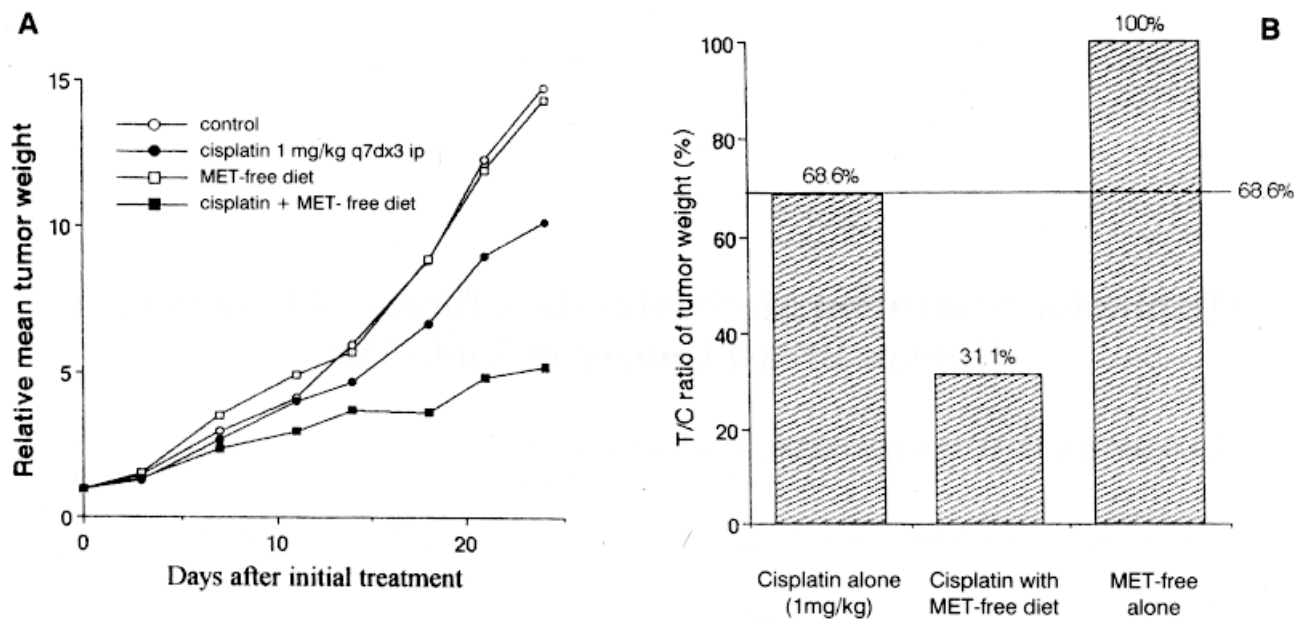


Figure 1. Synergistic antitumor activity of cisplatin and MET-free diet. Cisplatin (c-DDP) was administered IP at a dose of 1 mg/kg once a week for 3 weeks. The MET-free diet was given for 6 days each week with the control diet given on the seventh day. See text for details.

The defined diets, TD 93030 (MET-containing diet) and TD 92077 (MET-free diet) were purchased from TEKLAD (Madison, WI). Since normal cells can synthesize MET from homocysteine, the MET-free diet was also depleted of homocysteine and choline to allow extensive depletion of MET in the mouse serum. Mice were divided into four groups of five. Mice in the control and cisplatin only group were fed the MET-containing diet everyday. Mice in the MET-starved group and MET-starved/cisplatin group were fed the MET-free diet for six consecutive days each week followed by one day on the MET-containing diet each week.

Cisplatin was purchased from Bristol-Myers Squibb K. K., Tokyo. Mice in the cisplatin-only group and MET-starved/cisplatin group were treated with cisplatin intraperitoneally at a dose of 1 mg/kg on the fourth day after MET-starvation for three consecutive weeks.

The length and width of the tumors were measured with sliding calipers twice a week. The body weight of the mice was measured at the same time. The tumor weight was estimated according to the following formula:

$$\text{Tumor weight (mg)} = \text{length (mm)} \times [\text{width (mm)}]^2 / 2$$

If the mice showed signs of distress, they were sacrificed and the tumors were resected to measure the actual tumor weight. Antitumor efficacy was evaluated by the T/C ratio (%), where T was the actual tumor weight of the treated group and C was the actual tumor weight of the control group at sacrifice. The combination therapy was considered to be synergistic when the T/C ratio of the combination of cisplatin and the MET-free diet was lower than the value of (T/C ratio of cisplatin alone) X (T/C ratio of MET-free diet alone). Statistical analysis of the data was performed using the Student's-t test for unpaired samples. Mice in the cisplatin only group and in the cisplatin and MET-depletion group were sacrificed 3 hours after the fourth administration of cisplatin. The tumors were resected and frozen in liquid nitrogen as quickly as possible. The intra-tumoral concentration of total platinum in the samples was assessed by flameless atomic absorption spectrophotometry (8).

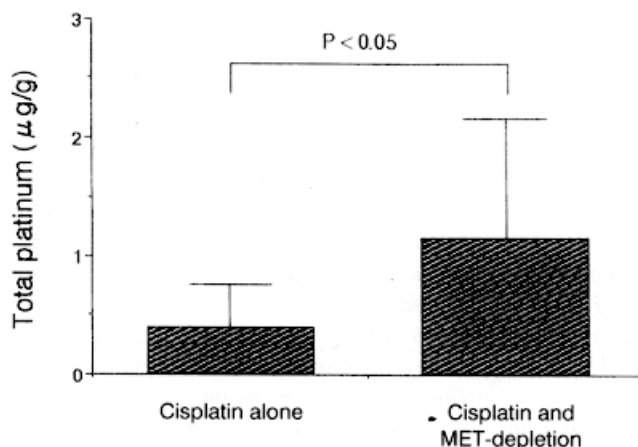


Figure 2. Total platinum concentration in MX-1 in nude mice. Mice in the cisplatin alone group and in cisplatin and MET-depletion group were sacrificed 3 hours after the fourth administration of cisplatin.

Results and Discussion

The T/C ratios of cisplatin only, MET-free diet only and cisplatin in combination with the MET-free diet against MX-1 were 68.9%, 94.4%, and 31.2%, respectively (Figure 1A and B). Thus, MET-starvation more than doubled the antitumor efficacy of cisplatin with statistical significance of $p < 0.05$. Thus, the modulating effect of MET-depletion on cisplatin efficacy was highly synergistic.

The intratumoral platinum concentration was higher in combination with methionine depletion than cisplatin-only and was statistically significant ($p < 0.05$) (Figure 2). It has been reported that pretreatment of buthionine sulfoximine (BSO) enhanced the antitumor activity of cisplatin (9). BSO is thought to decrease the glutathione (GSH) level, which allows increases in the total platinum concentration in cisplatin-treated tumors and enhanced antitumor activity of cisplatin. MET is a precursor of cysteine which is a component of GSH itself. MET-depletion has been previously shown to result in a decrease of intratumoral GSH (10). Thus, MET-depletion modulation of cisplatin may be due to decreased GSH levels. On the other hand, work from Scanlon's laboratory (11) has shown that low concentrations of cisplatin can inhibit the uptake of methionine in L1210 cells. The synergy observed in the present work could also be due to cisplatin modulating methionine depletion by inhibiting the methionine still remaining in the serum originating from tissue methionine biosynthesis and protein turnover from entering tumor cells, thereby further depleting tumor methionine stores.

Clinical studies have shown that a methionine-depleted total parenteral nutrition solution modulates efficacy of mitomycin C and 5-FU in patients with stage IV gastric carcinoma (12, 13). Thus methionine depletion has clinical efficacy. Future experiments will utilize the enzyme methioninase to further decrease serum methionine to essentially total depletion levels (14, 15) to increase cisplatin modulation and modulation of other drugs in preclinical and subsequent clinical studies.

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