Reduction of Streptozotocin Toxicity by 3-O-Methyl-D-glucose with Enhancement of Antitumor Activity in Murine L1210 Leukemia

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SUMMARY

3-O-Methyl-D-glucose (3-OMG), a nontoxic nonmetabolizable derivative of glucose, is effective in reducing the toxicity of streptozotocin (SZ). In mice the administration of 3-OMG prior to SZ increased the dose that killed 50% of the animals from 240 to 340 mg/kg. Furthermore, the combination of 3-OMG plus nicotinamide (also effective in reducing SZ toxicity) increased the dose that killed 50% of the animals to 540 mg/kg. In L1210 leukemic mice treated with SZ, there was a 2-fold increase in the median survival of animals pretreated with 3-OMG and a 3-fold increase in that of animals pretreated with the combination of 3-OMG and nicotinamide. Neither 3-OMG nor nicotinamide alone enhanced the survival of the leukemic mice. Pretreatment of normal mice with 3-OMG partially prevented the expected fall in hepatic nicotinamide adenine dinucleotide content. This study suggests that 3-OMG, by protecting normal tissue, will permit the administration of larger therapeutic doses of SZ in leukemic L1210 mice. The protective effect of 3-OMG against SZ toxicity appears to be partially mediated through conservation of the nicotinamide adenine dinucleotide content in the tissue.

INTRODUCTION

SZ, the N-nitroso-N-methylurea derivative of 2-deoxy-D-glucose, was isolated from the fermentation broth of Streptomyces achromogenes in 1960 (8, 17). It has been found to possess antitumor (14), oncogenic (11), and diabetogenic (12) properties. The diabetogenic activity of SZ has been correlated with a reduction in NAD concentration in the islet with subsequent β-cell necrosis (1, 13). Pretreatment with nicotinamide prevents the reduction of NAD content in the islet and the subsequent development of the SZ-induced diabetes (13, 16).

The glucose moiety of SZ facilitates the uptake of its cytotoxic group 1-methyl-1-nitrosourea into islets (1). Recently, prior administration of 3-OMG, a nontoxic nonmetabolizable derivative of glucose, has been shown to protect rats against the development of SZ-induced diabetes (5). We report the effect of 3-OMG pretreatment on the lethal and diabetogenic doses of SZ in normal mice and the effect of combination chemotherapy with 3-OMG and 3-OMG plus nicotinamide on the therapeutic response of SZ in experimental L1210 lymphocytic leukemia.

MATERIALS AND METHODS

Chemicals. SZ (U-9889, Lots 11837-GGS-22B and 105118-GGS-37A, observed to have optical rotation equivalent to 90% of the α anomer) was obtained from The Upjohn Co., Kalamazoo, Mich., and prepared as previously reported (5). 3-OMG, nicotinamide, alcohol dehydrogenase, and analytical reagents and buffers were obtained from Sigma Chemical Co., St. Louis, Mo.; all were of reagent grade.

Toxicity and Antitumor Assays. DBA/2 and C57BL x DBA/2 F1 (hereafter called BD2F1) male mice, 6 to 8 weeks of age, were obtained from The Jackson Laboratory, Bar Harbor, Maine. All injections were i.p. unless otherwise stated.

Antitumor activity was determined by measuring the ILS in mice given $10^6$ L1210 lymphocytic leukemia cells as compared to sham-injected controls. Assay procedures were identical to standard National Cancer Institute protocols (6). In all experiments the treatment was given daily for 5 days, beginning on the day after tumor inoculation.

SZ was prepared immediately prior to use, whereas 3-OMG and nicotinamide were dissolved in 0.9% NaCl solution 24 hr prior to administration as previously described (5). Injection volumes varied according to animal weights and ranged from 0.1 to 0.25 ml. 3-OMG was administered 10 sec prior to SZ, whereas nicotinamide was given 10 min prior to SZ. The percentage of ILS was calculated according to the formula

$$\% \text{ ILS} = \left( \frac{t}{c} - 1 \right) \times 100$$

where $t$ is the median survival time in days of the treatment group and $c$ is the median survival time of the control group.

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Analytical Procedures. Blood samples for glucose determination were obtained by retroorbital bleeding, and 100-μl samples were analyzed on a Beckman analyzer by the glucose oxidase system (5).

The NAD contents in liver and tumor cells were determined after sacrificing the animals by cervical dislocation. Livers were rapidly excised; excess blood was removed and immediately homogenized in a 1.5 (w/v) solution of 0.6 M perchloric acid at 4°C. Ascitic fluid was centrifuged at 800 × g for 5 min, and the pellet was separated and homogenized. The homogenate was centrifuged at 500 × g for 5 min, and the supernatant fluid was assayed directly for NAD by the method of Klingenberg (9).

RESULTS

Protection against SZ Diabetes. Plasma glucose concentrations were determined 72 hr after a single administration of SZ at doses of 0 to 300 mg/kg i.v. in overnight-fasted DBA/2 mice (n = 10). SZ produced no significant increase in glucose levels when compared to controls below 80 mg/kg (Chart 1). Above 100 mg/kg, SZ produced a progressive increase in the mean plasma glucose; at 120 mg/kg the mean plasma glucose level was 450 mg/100 ml. When the mice were pretreated with 3-OMG (2.0 g/kg), the optimal dose) prior to SZ, there was no increase in plasma glucose concentration until the SZ dose was greater than 180 mg/kg.

Protection against SZ-induced Lethality. Chart 2 depicts the effect of various doses of SZ on 30-day survival (n = 10). Pretreatment with 3-OMG (2.0 g/kg) increased the LD_{50} of a single injection of SZ from 240 mg/kg to 340 mg/kg. Nicotinamide (500 mg/kg) provided similar protection. The combination of 3-OMG (2.0 mg/kg) and nicotinamide (500 mg/kg) increased the LD_{50} of SZ to 540 mg/kg.

Tumor Studies. BD2F1 mice were given injections of 10^5 L1210 leukemic cells on Day 0 and divided into experimental groups (n = 10). On each of the following 5 days, each group was subjected to one of the following regimens: (a) control; (b) 3-OMG (2.0 mg/kg); (c) nicotinamide (500 mg/kg); (d) citrate buffer; (e) varying doses of SZ; (f) 3-OMG (2.0 mg/kg) plus SZ; (g) nicotinamide (500 mg/kg) plus SZ; (h) 3-OMG plus nicotinamide plus SZ.

Chart 3 shows the effect on survival of the various regimens. Although not shown the administration of citrate buffer, 3-OMG, or nicotinamide in the absence of SZ administration did not prolong the survival compared to untreated leukemic mice. When given SZ alone, doses above 70 mg/kg resulted in a negative ILS value, indicating toxic deaths. Pretreatment with 3-OMG increased the therapeutic response to SZ (p < 0.01), and the animals were able to tolerate a higher dose of SZ. Pretreatment with nicotinamide produced effects similar to those of 3-OMG. The optimal dose of SZ, prior to varying the doses of SZ, increased from 70 mg/kg, when SZ was given alone, to 100 mg/kg, when 3-OMG was given prior to SZ, and further increased to 150 mg/kg by pretreatment with both nicotinamide and 3-OMG.

Effect of Pretreatment on Hepatic NAD Content. Since previous studies had shown that SZ reduced the NAD content of hepatic and tumor cells (13), the effect of pretreatment by 3-OMG on the NAD levels was evaluated in our mice. Normal DBA/2 mice were given either varying doses of SZ or 3-OMG (2.0 mg/kg) or a combination of SZ and 3-OMG by i.v. injection. The animals were sacrificed 4 hr after injection, and hepatic NAD content was determined.

Table 1 summarizes the results of these studies. In normal mice the hepatic content of NAD decreased with the increasing doses of SZ. Pretreatment with 3-OMG resulted in...
a slightly higher NAD content at each SZ dose level. Statistical significance was observed only at 50 and 200 mg SZ per kg. At 100 mg SZ per kg, by using increasing doses of 3-OMG, a significant conservation of hepatic NAD content was apparent.

**DISCUSSION**

Although tissue selectivity in cancer chemotherapy is of paramount importance, it is relatively recent that enhancement has been achieved pharmacologically by drug interactions, e.g., methotrexate-citrovorum factor, methotrexate-thymidine (4). Our results suggest that the therapeutic efficacy of SZ can be enhanced by pretreatment with both 3-OMG and 3-OMG plus nicotinamide. The optimal dose for a chemotherapeutic agent usually approximates the lethal dose that kills 10% of the animals for a given treatment schedule (7). Since the optimal dose of SZ could be substantially increased by pretreatment with 3-OMG and/or nicotinamide without loss of therapeutic effectiveness in the L1210 leukemic mice, we would conclude that 3-OMG and nicotinamide decreases the host susceptibility to SZ.

The basis for the protective effect demonstrated by 3-OMG is unknown. SZ and 3-OMG are structurally related in that both are 6-carbon sugars, similar, stereochemically, to glucose itself. The observation that the protective effect of 3-OMG is additive to that exhibited by nicotinamide suggests that 3-OMG and nicotinamide operate at least partially through different mechanisms.

Supporting this view are studies on the ability of these 2 agents to block the diabetogenic activity of SZ. For example, 3-OMG is maximally protective when administered immediately prior to SZ injection (5), whereas nicotinamide exerts a protective effect when given up to 2 hr after injection of SZ (3, 8, 10). The differential effect between normal and tumor tissue might be the result of the relatively greater degree of anaerobic glycolysis present in the L1210 cells. Therefore, at a given ratio of SZ to 3-OMG, normal tissue may be better protected than tumor cells from metabolic effects of SZ.

Finally, since the therapeutic use of SZ in humans has been limited by renal toxicity (15) and since 3-OMG is entirely excreted by the kidneys (probably by filtration and lack of tubular reabsorption), its potential effect on limiting renal toxicity is intriguing (2). Currently, experiments in rats are underway to resolve this question.

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**REFERENCES**

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