Inhibitory Effect of 2-Deoxy-d-glucose on Liver Tumor Growth in Rats

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ABSTRACT

Since tumor cells are more dependent on glycolysis for energy supply than other cells, we tested whether its inhibition by 2-deoxy-d-glucose (2-DG) affects tumor growth. Male Wistar rats were inoculated in the liver with tumor cells from a chemically induced colon adenocarcinoma. From day 5 after inoculation 2-DG (400 mg/kg/24 h) was continuously infused into the hepatic artery for 5 days; controls received saline in the same fashion. Seven days after the end of infusion, the animals were sacrificed. A second experimental group of rats was treated with isolated liver perfusion for 30 min with oxygenated blood through the portal vein and hepatic artery simultaneously. In the perfusate, 400 mg/kg 2-DG were added, and the rats were sacrificed at 10 days after perfusion. A first control group underwent perfusion without 2-DG, and a second control group received i.v. infusion of 2-DG (400 mg/kg/30 min) for 30 min over 5 days. A nontreated control group was also added. All animals survived the procedures. The concentration of blood glucose increased in the rats receiving 2-DG i.v. and intraarterially but was unchanged in the other groups. The tumor growth was significantly reduced by 2-DG in all experimental groups, with no difference between the groups. It is therefore concluded that 2-DG is of potential interest in the treatment of malignancies. Since local application of 2-DG avoids the risk for systemic side effects, this approach should be explored further.

INTRODUCTION

A characteristic of growing tumor cells is their capacity to sustain high rates of glycolysis under aerobic conditions (1-3). This difference in energy metabolism between tumor tissue and normal tissue has been utilized successfully in the development of new diagnostic imaging techniques (4, 5), and might also be used in the therapy of malignant tumors. For example, administration of a glucose precursor-deficient diet has been shown to reduce tumor growth in experimental systems (6), and administration of glucose analogues, such as 2-deoxy-d-glucose, causes a marked glycolytic inhibition of tumor growth and a modest prolongation of survival time in animals with experimental tumors (7). It has also been proposed that hypoglycemia in combination with glycerol infusion might be used as an antineoplastic therapy (8). However, the systemic use of glucose analogues is limited by their systemic actions, such as enhancement of plasma levels of insulin, glucagon, and catecholamines (9-11) and inhibition of thyroid hormone secretion (12). This drawback might be circumvented by the use of regional administration. We therefore investigated the effect of regional administration of high doses of 2-DG3 either infused in the hepatic artery or administered in an isolated in vivo perfusion of the liver in rats with tumor growth limited to the liver.

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3 The abbreviation used is: 2-DG, 2-deoxy-d-glucose.

MATERIALS AND METHODS

Male inbred Wistar-Furth rats (Anticimex Laboratories, Solna, Sweden) weighing 300-330 g were used. The animals were housed 3/cage and had access to rat chow (Astra-Ewos R3) and water ad libitum. The light was on between 7 a.m. and 7 p.m.

The experiment was approved by the Animal Ethics Committee at Lund University and adhered to the Guiding Principles in the Care and Use of Animals.

The tumor used in the experiment was a N-methyl-N-nitrosourea-induced adenocarcinoma (NGW) of the colon (13). The tumor was propagated intraabdominally every seventh day. The number of passages before the generation used in this study was 20 (hepatic artery infusion) and 27 (perfusion). The condition of the tumors was controlled at regular intervals for occurrence of infection and alteration in tumor growth.

The tumors from the abdominal cavity were dissected and cut into small fragments. Trypsin-E-DTA solution was added and mixed for 25-40 min. After centrifugation, the supernatant was removed. Trypsinisation was stopped by inactivated rat serum.

After adding trypan blue, vital cells were counted in a Buchtner chamber (14). The final cell solution was then diluted to a suspension containing 1.0 x 10⁶ viable tumor cells/0.1 ml. Within 1 h after the preparation, 0.1 ml of the tumor cell suspension was inoculated into the periphery of the central lobe of the liver beneath the liver capsule. Access to the liver was achieved through a midline incision under chloral hydrate anesthesia (25 mg/100 g body weight). In order to avoid leakage of tumor cells from the injection site after withdrawal of the injection needle, a Spongostan sponge was pressed against the opening until hemostasis was complete.

Seven days after tumor inoculation, the abdomen was opened in the midline under clean but not sterile conditions under light ether anesthesia. The tumor volume in the liver was assessed by the method of Carlson et al. (15). Thereafter, rats were randomized to different treatment protocols. Two separate experiments were performed.

Infusion of 2-DG in the Hepatic Artery. The hepatic artery was cannulated with a polyethylene catheter (PE 10) as described previously using a Zeiss operating microscope (×12). The catheter was brought out at the neck, infusion was started, and the animals were placed in metabolic cages. The animals were randomized to receive either 2-DG or saline.

2-DG (Sigma Chemical Co., St. Louis, MO) was dissolved in saline and infused at a dose of 400 mg/kg/24 h in 11 rats. The rate of infusion was 1 ml/h. The infusions were carried out continuously for 5 days. At the termination of infusion, venous blood was drawn. The intraarterial catheter was removed. The animals were followed for another 7 days and then sacrificed under ether anesthesia.

Controls. Ten control rats received an infusion of saline at the same rate. They were then handled as above.

In Vivo Perfusion of the Liver. The isolated perfusion of the liver was performed as described in detail elsewhere (16). Rats were intubated under ether anesthesia and placed on a heating pad. They were connected to a respirator giving 35% N₂O, 65% O₂, and 0.7% halothane in a volume of 0.4 liter/min at 85 breaths/min. A silicone cannula was placed in the jugular vein for infusion. Before opening the abdomen, an infusion of Ringer's solution with the addition of 25 mg glucose/liter and 0.16 mmol NaHCO₃ was started, and 12 ml were administered. A Portex cannula was placed in the tail artery for sampling. The abdomen was opened through a midline incision. The liver was then isolated and perfused for 30 min. The hepatic artery was perfused with a Watson-Marlow pump (type 502s) in a pulsatile flow of 5.3 ml/min and the portal vein with a Watson-Marlow pump (type MHRE 72) in a continuous flow of 24.2 ml/min. The perfusion medium...
contained 9 ml blood from unoperated rats of the same strain, 5 ml Ringer-acetate, and 0.3 mmol NaHCO₃ and was kept at 42.0°C in a water bath reservoir. It was oxygenated with the help of a simple oxygenator. The animal was heparinized with 50 units (heparin; KabiVitrum, Stockholm, Sweden). After 30 min all cannulas were removed, and the rat was given 2.5 ml Protamin (KabiVitrum) i.v. The abdominal wound was closed, and the respirator was adjusted to 100% O₂. The duration of vena caval and aortic occlusions did not exceed 40 min, although this time is probably not critical. The time required to complete the whole procedure varied between 60 and 70 min.

Eleven rats underwent perfusion for 30 min with 2-DG at a dose of 400 mg/kg body weight added in the perfusate.

Controls. Ten rats underwent an identical perfusion but without 2-DG. Another group of 10 rats was given an i.v. infusion daily for 30 min of 2-DG at 80 mg/kg body weight for 5 days (total dose, 400 mg/kg body weight). Ten rats served as controls, with an infusion of NaCl.

All experiments were run in pairs for intraarterial infusion and quadruples for in vivo perfusion.

Tumor volume was again estimated at sacrifice, and liver and tumor tissue were taken for histology. The histological specimens were stained with hematoxylin-eosin.

Body weight and liver function tests were measured. Blood concentration of glucose and plasma insulin levels were assayed before the start of administration of 2-DG, immediately after and at sacrifice.

Glucose was estimated according to glucose-oxidase method (17) and insulin with a radioimmunoassay using rabbit anti-porcine insulin antiserum and rat insulin as standard (18).

Statistical evaluation was performed using the Kruskall-Wallis test.

RESULTS

All animals survived the experiment. There was no obvious difference in the behavior of the animals after the experiment. There was a small drop in the body weight in all groups at sacrifice compared to weight at operation (Table 1).

Blood glucose levels rose after administration of 2-DG in the hepatic artery and i.v. (Table 2). In the other groups with isolated perfusion no change in blood glucose levels was seen.

Plasma insulin levels increased after intraarterial as well as i.v. administration of 2-DG (Table 3) but was unchanged in the other groups.

The liver function tests at sacrifice are seen in Table 4 from groups with perfusion and i.v. delivery. There were no significant alterations.

The tumor volumes before the start of therapy were smaller in the first experimental (intraarterial) than the second (perfusion and i.v.) experimental series. However, there were no differences between experimental and control groups before the start of therapy. At sacrifice the tumor volume in the saline-infused intraarterial group was larger than in the 2-DG group (Table 5).

In the other experimental series, tumor growth was also retarded in groups receiving 2-DG either in perfusion or i.v. systemic infusion. Tumor growth in the sham group was smaller than that in the control group with i.v. infusion of saline alone.

The histological examinations of the tumors showed a normal hepatic parenchyma with intrahepatic tumor with widespread necrosis but without any differences between the groups.

DISCUSSION

Glucose analogues, such as 2-DG, have been found to profoundly inhibit glucose metabolism in cancer cells in vivo as well as in vitro (7, 19–21). 2-DG has been used in different experimental tumor systems, and retardation of local tumor growth has been found (7). This effect seems to be more readily demonstrable than prolongation of survival. This might be expected since the drug has nonspecific systemic effects on the host, such as weight loss, weakness, seizures, and edema, which could affect the survival time (22).

In this study, we examined the feasibility of using 2-DG in a locoregional application in the liver. We chose the systemic dose based on studies of insulin release (10), and by giving it either in the hepatic artery or in an in vivo isolated liver perfusion system, we achieved a high dose in the tumor tissue. There were no adverse systemic effects observed, and blood glucose and plasma insulin levels were unchanged after the application of 2-DG in the isolated perfusion system but rose after i.v. and intraarterial delivery. Thus, one-fifth of the dose (80 mg/kg body weight) given i.v. was apparently not toxic but caused transient hyperglycemia and hyperinsulinemia. The dose of 400 mg/kg body weight was well tolerated when given in the hepatic artery, but there was some effect on peripheral glucose and insulin levels, and there may thus have been an incomplete extraction of 2-DG during the delivery in the liver. The isolated in vivo perfusion with 2-DG completely avoided peripheral effects on glucose and insulin levels.

Tumor growth was retarded in all groups receiving 2-DG when studied 7 days after administration. The tumor volumes in the intraarterial experiment were smaller than those in the perfusion experiment, and this makes comparison between the experiments difficult. The mechanisms by which 2-DG inhibits the tumor growth rate may be several, such as inhibition of glucose uptake (19), impaired glycolysis at the phosphoglucoisomerase level (23), and inhibition of adeninonucleotide and phosphate metabolism (24). 2-DG is also incorporated into glycoprotein components, resulting in cell surface changes. The possible mechanism of action of interference with the energy supply of the tumor tissue is of particular interest, since it is well known that metastatic deposits in the liver are mainly supplied by the hepatic artery (25).

It is thus apparent that 2-DG has an inhibitory effect in this tumor. By applying 2-DG in an isolated perfusion system, we
REFERENCES

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The references cited include:

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