Survival of Pleomorphic Sarcoma-37 Transplanted Virgin Female DBA/2J Mice: Effects Produced by High Blood Glucose Levels Alone and in Combination with Drugs

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Abstract □ Procedures for producing, monitoring, and maintaining 3-hr 400–500-mg % whole blood glucose levels in Sarcoma-37 (S-37) transplanted virgin female inbred DBA/2J mice are described. Aqueous infused 40% (w/v) dextrose was evaluated as a potential therapeutic factor. The effects of procedural variations on treatment survival and longevity are discussed. One hundred percent infusion safety and 25% increased longevity over nontreated transplanted mice were achieved. Doxorubicin and/or dacarbazine were also evaluated when administered prior to the dextrose infusion. Doxorubicin followed by intravenous dextrose increased survivor longevity by 37%, but this combination was unsafe at the drug dosages employed. A large dose of dacarbazine was safe and effective alone but unsafe when given prior to the infusion, although the survivors lived 29% longer than the untreated transplanted controls. Both drugs were marginally effective, but safe, when given together. When given together prior to the infusion, only 87% survived the treatment. The survivors lived 6 days longer than the controls.

Keyphrases □ Blood glucose—high levels, alone and with doxorubicin or dacarbazine, effect on survival of tumor-transplanted mice □ Dextrose infusion—alone and with doxorubicin or dacarbazine, effect on survival of tumor-transplanted mice □ Doxorubicin—combined with high blood glucose levels, effect on survival of tumor-transplanted mice □ Dacarbazine—combined with high blood glucose levels, effect on survival of tumor-transplanted mice □ Antineoplastics—doxorubicin and dacarbazine, alone and with high blood glucose levels, mice

The selectively higher aerobic glycolytic rate noted in cancerous tissue as opposed to normal tissue was reported by Warburg in 1930 (1). This finding is controversial (2), although it has been demonstrated (3, 4) that dextrose levels of 400 mg/100 ml of whole blood cause a pH differential of as much as 1 unit to exist only a few millimeters apart in normal *versus* malignant tissues of the same organ. Tumor remissions were reported following the maintenance of hyperacidic tumor tissue (5).

Cancerous mice, stabilized at 400-mg % blood glucose levels, exhibit slower tumor growth, more remissions, and greater longevity than untreated mice (6). Maintenance of 400-mg % dextrose-induced hyperglycemia assures equilibration between tissues and high target tissue acidity. Although the pH of both tumor and normal tissues drops, the acidity of the former is greatest. Lysosomal enzymes, more active at lower pH's, catabolize the malignant tissue preferentially. The less acidic normal tissue provides a poorer substrate for the lysozymes, and the "self-destruction" stops according to this hypothesis.

It was suggested (6) that a "triggering attack" by one or more agents be employed in addition to dextrose infusion, since higher remission rates are desirable. X-Irradiation, massive doses of vitamin A, and cell cycle-specific or nonspecific antineoplastic agents disrupt the DNA and/or protein synthetic sequence, weakening lysosomal and cellular membranes. In theory, the application of several membrane-weakening agents with different mechanisms of action, given together, could produce additive, if not synergistic, beneficial results.

This study was designed to: (*a*) create an easily duplicated procedure for producing, monitoring, and maintaining safe 3-hr 400–500-mg % whole blood glucose levels in Sarcoma-37[1] (S-37) transplanted DBA/2J mice; (*b*) determine whether a causal relationship exists between this treatment and mouse longevity; and (*c*) evaluate whether doxorubicin (I) and/or dacarbazine (II), administered prior to the dextrose infusion, alter safety and longevity results. This investigation utilized known active mouse (7) and human (8) antisarcoma agents I and II. Tumor size, shape, and growth characteristics were observed.

EXPERIMENTAL

The following materials were used: genetically standardized inbred strains of virgin female DBA/2J mice with in-host S-37 transplanted tumors[2]; an infusion pump with variable rate settings and interchangeable holders for different syringe capacities[3], syringes[4]; polyethylene tubing[5]; 26-gauge 12.7-mm hypodermic needles[6]; a reflectance colorimeter[7]; glucose oxidase reagent strips[8]; capillary tubes (1.12–1.17 mm i.d.); I and II[9]; 40 % (w/v) aqueous dextrose; aqueous pentobarbital sodium, 32 mg/10 ml; and normal saline.

Virgin female DBA/2J mice, 15–20 g, were transplanted with 1 mm^3 of vascular S-37 tumor tissue[10]. They were treated 6 days later; chemotherapeutic agents were administered 15 min prior to anesthesia. Compound I was administered subcutaneously, II was given intraperitoneally, and 80 mg of pentobarbital sodium/kg was given with an oral syringe. Anesthetized mice were secured, and the tails were immobilized.

An infusion pump syringe holder was adapted to contain six 1-ml syringes. Each syringe was connected to 1 m of polyethylene tubing by a 26-gauge 12.7-mm needle. Another needle of the same size was broken near the syringe adaptor end, and this end was fitted into the far end of the tubing. The beveled end of the needle was then inserted into the mouse tail vein. The six syringes, prefilled to 0.5 mg with 40 % (w/v) aqueous dextrose, were activated at a slow setting (No. 19-21) on the pump.

Postorbital venous blood samples were periodically taken with a capillary tube. Blood glucose levels were determined by comparing the glucose oxidase strip color with chart standards (Cage 2 in Table I) or by reading the strip in a reflectance colorimeter (Cages 3–9 and 13 in Table I). Levels of 450 mg % were usually obtained in 60 ± 15 min (~0.2 ml of infusate). The infusion was discontinued at this time, and blood sampling was continued for another 2 hr. If the level fell below the desired 400 mg %, the infusion was reinstated. To prevent blinding the mice, no more than three samples were taken from behind either eye. A limit of four samples per mouse prevented too great a reduction in blood volume.

The dextrose original method (Cage 2) involved a 1:2 blood to saline

[1] R. E. Orth, unpublished data.
[2] Jackson Laboratories, Bar Harbor, ME 04609.
[3] Model 975, Harvard Apparatus Co., Millis, MA 02054.
[4] Plastipak disposable sterile tuberculin syringe 5602, 1 ml-TB, Becton-Dickinson and Co., Rutherford, NJ 07070.
[5] PE 20 (7407), Intramedic, Animal Tested Medical Formulation, Clay Adams Co., Parsippany, NJ 07064.
[6] B-D Yale hypodermic needle, Luer-lok hub, sterile, disposable, *stainless steel* cannula, Becton-Dickinson and Co., Rutherford, NJ 07070.
[7] Reflectance meter, Ames Co., Elkhart, IN 46514.
[8] Dextrostix, Ames Co., Elkhart, IN 46514.
[9] Doxorubicin (NSC 123,127) was supplied as the hydrochloride in 10-mg vials, and dacarbazine (NSC 45,388) was packaged in vials of 100 mg (Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014).
[10] Macerated S-37 tumor obtained from 7-day serial transplants.

Table I—Hyperglycemic and Drug Treatment of S-37 Transplanted DBA/2J Mice

Cage	Treatment	Fraction Surviving Treatment[a]	Longevity beyond Transplant Date[b]
1	Controls[c]	78/78	26.3 ± 1.8
2	Dextrose original method	48/62	26.1 ± 2.3[d]
3	Dextrose first modification	19/19	28.1 ± 1.3[d]
4	Dextrose second modification	16/16	31.2 ± 2.6
5	Dextrose third modification	36/36	32.8 ± 2.2
6	Dextrose second modification plus I, 5 mg/kg sc	8/16	36.0 ± 5.6
7	Dextrose second modification plus I, 2.5 mg/kg sc	11/16	35.9 ± 10.8
8	Dextrose second modification plus II, 200 mg/kg ip	12/16	33.8 ± 0.3
9	Dextrose second modification plus II, 100 mg/kg ip	16/16	30.2 ± 6.3
10	Isotonic saline plus I, 5 mg/kg sc	12/12	30.1 ± 2.6
11	Isotonic saline plus II, 200 mg/kg ip	12/12	31.5 ± 2.6
12	Isotonic saline plus I, 2.5 mg/kg sc plus II, 100 mg/kg ip	16/16	28.5 ± 2.3
13	Dextrose second modification plus I, 2.5 mg/kg sc plus II, 100 mg/kg ip	14/16	32.1 ± 2.4

[a] Includes deaths occurring within 24 hr of treatment. [b] The mean and the standard error of the mean in days surviving transplantation with 95% confidence. [c] Obtained from Cages 2–13 following transplantation prior to treatment. [d] Insignificant change in longevity from control values of Cage 1 (Student two-tailed t test with 95% confidence).

dilution and then placement of this mixture on the glucose oxidase reagent strip 1 min before washing and reading. The results were compared with a color chart on the reagent strip container. Data collected by reading the strips in a reflectance colorimeter were obtained by the dextrose first modification procedure (Cage 3). These mice were infused with aqueous dextrose immediately upon immobilization while still unconscious. This method required no dilution of the fresh venous blood samples, since readings up to 1000 mg % were possible using the expanded scale of the colorimeter. The dextrose second modification procedure (Cage 4) introduced a time lapse between immobilization and treatment. Therapy was commenced only when the mice were conscious.

These first three procedures rely on tail vein intravenous aqueous dextrose administration. The dextrose third modification (Cage 5) varied from the second modification procedure only by the route of administration. The intraperitoneal method was adopted in this latter infusion technique. The control mice (Cage 1) were anesthetized, secured, and infused with 0.2 ml of isotonic saline for 1 hr. The dextrose second modification method was utilized in all combination therapy with drugs.

RESULTS AND DISCUSSION

Untreated control mice lived 26.3 days beyond the S-37 tumor transplantation date (Cage 1). Twenty-three percent of the mice subjected to the dextrose original method died during treatment (Cage 2). The longevity for survivors in Cage 2 was about the same as for the Cage 1 controls. This dextrose monitoring procedure, employing a blood–saline dilution technique coupled with naked eye reagent strip color comparison with chart standards, was ineffective. The technique yielded false low values, resulting in extremely high actual blood glucose levels. More accurate assessments of undiluted blood glucose concentrations were obtained with the three following procedural modifications. An expanded scale reflectance colorimeter was utilized in which readings up to 1000 mg % were possible.

The dextrose first modification procedure (Cage 3) differed from the original method only by utilization of the colorimeter. Mice were unconscious at the time the infusion was initiated in both procedures. A safe treatment (100% survival) and a small but insignificant increase in longevity were observed. If, as in the dextrose second modification procedure (Cage 4), the sugar infusion was commenced only after the animal regained consciousness, the treatment yielded a safe and a significant 5-day increase in life expectancy over the control group.

The last variation of the infusion procedure was administration of the 40% (w/v) aqueous dextrose intraperitoneally (Cage 5). This dextrose third modification method resulted in a 100% safe treatment and a significant 6-day increase in longevity over the controls.

Obtaining and maintaining 400–500-mg % blood glucose levels was more difficult intraperitoneally than intravenously. The longevity differences obtained by these two procedures were statistically insignificant. Therefore, the more easily regulated intravenous dextrose second modification method is preferred and was used with drug therapy (Cages 6–9 and 13).

A 5-mg/kg sc dose of I (Cage 10) was safe and effected a significant 4-day increase in transplanted mouse lifespan. When I was given with infused dextrose, the treatment was unsafe, but the survivors of either 2.5 (Cage 6) or 5 (Cage 7) mg/kg lived 10 days longer than the controls. Although hyperglycemic treatment with I yielded significant longevity increases, the great deviations from the mean longevity and poor treatment survival rates did not favor adoption of this therapy.

A 200-mg/kg ip dose of II (Cage 11) was safe and resulted in a significant 5-day increase in longevity over the controls. If this same dose of II was administered with the dextrose treatment (Cage 8), the 75% surviving lived 7 days longer than did the control animals. By reducing the dose of II to 100 mg/kg prior to infusion with dextrose (Cage 9), survival was assured but therapy was minimally effective.

Given together, I and II provided safe but marginally effective therapy for the S-37 transplanted mice (Cage 12). However, when hyperglycemic treatment was added to the regimen (Cage 13), the mice survived an additional 6 days. Unfortunately, 12% of the mice died during treatment.

"Selective survival" may account, in whole or in part, for the increased longevity noted for mice given I and/or II during unsafe treatments (Cages 6–8 and 13). A toxic treatment may selectively kill the weakest mice, allowing the stronger animals to survive and live longer lives. The 26-day longevity values noted for both the controls and the unsafe dextrose original method survivors may provide evidence that conflicts with the selective survival concept.

SUMMARY

Safe procedures for producing, monitoring, and maintaining 3-hr 400–500-mg % whole blood glucose levels in S-37 transplanted virgin female inbred DBA/2J mice were achieved (Cages 3–5). Sarcoma-37 transplanted mouse longevity was significantly increased following a 100% safe treatment with a single incident of: 40% dextrose infusion (Cages 4 and 5), 5 mg/kg sc of I (Cage 10), 200 mg/kg ip of II (Cage 11), or 2.5 mg/kg sc of I with 100 mg/kg ip of II (Cage 12). Combinations of I and/or II with the dextrose may be less than 100% safe, but survivor longevity is increased (Cages 6–8 and 13).

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