Survival of Pleomorphic Sarcoma-37 Transplanted Virgin Female DBA/2J Mice: Hyperthermia and Hyperglycemia, Alone and in Combination with Drugs

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Abstract □ Sarcoma-37 (S-37) transplanted DBA/2J mice were submitted to a controlled whole body hyperglycemic–hyperthermic ("double attack") treatment. A single exposure to the 3-hr 400–500-mg % blood glucose level coupled with 1 hr of whole body 40.0° warming was safe and extended longevity by 40% over the nontreated controls. This increase suggests that additive or potentiated effects follow the two-parameter procedure. The treatment safety and resultant increased longevity were not enhanced by single-dose chemotherapy with doxorubicin and/or dacarbazine.

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

Nearly a half century ago, investigators attempting to characterize functional differences between normal and cancerous tissue from the same organ found a higher rate of aerobic glucose metabolism for the latter (1). von Ardenne (2) attempted to capitalize on the resultant higher intracellular lactic acid levels in glucose-stressed cancerous tissue. The premise is that the more acidic neoplastic tissue offers a better substrate for support of intrinsic catabolic lysosomal enzyme activity. A single safe 3-hr 400–500-mg % blood glucose level was followed by a significant increase in the longevity for Sarcoma-37 (S-37) transplanted virgin female DBA/2J mice (3).

A review on the association of heat and cancer (4) and data on whole body hyperthermia in humans (5) and animals (6) are available. One hour of whole body 40.0° warming was found to be a safe, statistically significant factor in prolonging the longevity of S-37 transplanted DBA/2J mice[1].

Doxorubicin (I) and dacarbazine (II), drugs known to interfere with cell membrane protein synthesis, were effective alone and in combination against human (7) and mouse (8) sarcomas *in vivo*.

A dextrose infusion–whole body warming technique was evaluated *in vitro* and *in vivo* in seven different rodent tumor types (9), and tumor diameter decreases were noted.

The effects of a single incident of 3-hr 400–500-mg % whole blood glucose levels, 1 hr of warming at 40.0°, 5 mg/kg sc of I, and 200 mg/kg ip of II were individually evaluated for safety and resultant longevity under controlled conditions in S-37 transplanted mice (Table I, cages 2–5). Various combinations of these four treatments also were evaluated (Table I, cages 6–10 and 12). Virgin female DBA/2J mice were the treatment and control subjects in cages 2–10 and 1, respectively. Transplanted male DBA/2J mice were subjected to control and dextrose–warming ("double attack") procedures (cages 11 and 12, respectively) to determine differences in responses by sex.

[1] R. E. Orth, H. J. Swidler, and M. S. Zarakov, unpublished results.

EXPERIMENTAL

Transplantation—An S-37 bearing donor mouse[2] was sacrificed 7 days after serial subcutaneous transplantation. The tumor was removed, moistened with isotonic saline, and minced. One cubic millimeter of tissue was placed in a 15-gauge trocar and administered to 15–20-g DBA/2J mice subcutaneously into the thigh 6 days before treatment.

Drugs—Whenever drugs were administered to experimental animals, isotonic saline was given to the paired control animal in the same way, at the same site, and in the same chronological sequence. When used, I[3] and II[3] were given 15 min prior to the hypnotic agent. Pentobarbital sodium, 80 mg/kg po, was administered (3.2 mg/ml of 1:2150 diluted B complex elixir). Aqueous dextrose (40% w/v) and isotonic saline were the infusion solutions used.

Dextrose Intravenous Infusion Procedure —Unconscious mice were secured with their heads down (shock position), on their backs, with immobilized tails. An adapted infusion pump[4] syringe holder contained six 1-ml syringes[5]. Each syringe was connected by tubing[6] to a 26-gauge 12.7-mm needle[7] for insertion into the tail vein of each of six mice. The syringes, filled with the dextrose solution[8], were activated at pump speed setting No. 19. Postorbital venous blood samples of approximately 0.05 ml were periodically obtained using heparinized capillary tubes[9].

Blood glucose levels were determined by reading glucose oxidase–peroxidase reagent strips[10] in a reflectance colorimeter[11]. Once 400–500-mg % levels were obtained, the infusion was slowed to a replacement rate with a pump setting of No. 20 or 21, and these levels were maintained for 2 hr.

Water Bath Procedures—Conscious mice were immersed to the neck in a water bath[12] for 1 hr at 40.0 ± 0.3°[13] (Table I, cages 3, 7–10, and 12) or 37.0 ± 0.3° (Table I, cages 1, 2, 4–6, and 11) following oral administration of 0.01 ml of B complex elixir. A stream of 95% O_2 was directed to the head of each animal during this period. Following the immersion, the animals were gently toweled and placed in a 37.0° oxygenated compartment for 1 hr before being returned to their cages.

[2] Sarcoma-37 tumor-in-host and genetically standardized inbred DBA/2J mice, Jackson Laboratory, Bar Harbor, ME 04609.

[3] Doxorubicin (NSC 123,127) hydrochloride and dacarbazine (NSC 45,388), supplied by Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014.

[4] Model 975, Harvard Apparatus Co., Millis, MA 02254.

[5] Plastipak disposable syringe 5602, sterile-tuberculin, Becton-Dickinson, Rutherford, NJ 07064.

[6] PE 20 (7407) Intramedic, Animal Tested Medical Formulation, Clay Adams Co., Parsippany, NJ 07064.

[7] B-D Yale hypodermic needle, Luer-lok hub, sterile disposable, stainless steel cannula, Becton-Dickinson, Rutherford, NJ 07070.

[8] Diluted 50% dextrose injection USP obtained from McGaw Laboratories, Division of American Hospital Supply Corp., Glendale, CA 91201.

[9] Van-Lab, yellow band with heparin, Catalog No. 15401-683, VWR Scientific Co., Phoenix, AZ 85001.

[10] Dextrostix, Ames Co., Elkhart, IN 46514.

[11] Reflectance meter, Ames Co., Elkhart, IN 46514.

[12] NAPCO bath model 87251 with Cenco 19245 0–100° thermometer with 0.1° divisions.

[13] Tele-thermometer model 43TA with appropriate sensor probes, Yellow Springs Instrument Co.

Table I—Dextrose, Heat, and Drug Treatment of Sarcoma-37 Transplanted Mice

Cage	Treatment[a]	Fraction Surviving Treatment[b]	Longevity beyond Transplant Date[c]
1	Female controls[d]	32/32	26.5 ± 0.7
2	Dextrose intravenous infusion[e]	12/12	31.4 ± 0.9
3	Whole body 40.0° warming[f]	12/12	30.1 ± 0.9
4	I, 5 mg/kg sc[d]	8/8	28.2 ± 1.8
5	II, 200 mg/kg ip[d]	8/8	32.2 ± 1.1
6	I, 2.5 mg/kg sc, plus II, 100 mg/kg ip[d]	8/8	29.4 ± 1.5
7	Dextrose intravenous infusion plus whole body 40.0° warming (double attack)[g]	8/8	37.0 ± 1.1
8	I, 2.5 mg/kg sc, plus double attack[g]	7/8	36.3 ± 1.5
9	II, 100 mg/kg ip, plus double attack[g]	8/8	35.2 ± 1.1
10	I, 2.5 mg/kg sc, plus II, 100 mg/kg ip, plus double attack[g]	6/8	31.5 ± 1.6
11	Male controls[d]	16/16	30.8 ± 0.6
12	Male double attack[g]	24/24	33.2 ± 0.9

[a] All mice were anesthetized with pentobarbital sodium, 80 mg/kg po, prior to immobilization. [b] (Number surviving treatment + 24 hr)/(number of mice treated). [c] Days of life between transplant date and death date (mean ± *SEM*). [d] Isotonic saline tail vein 3-hr 0.5-ml iv infusion. Whole body 37.0° water bath immersion during the 3rd hr. [e] Forty percent (w/v) dextrose tail vein intravenous infusion at rate that maintains 3-hr 400–500-mg % blood glucose level. Whole body 37.0° water bath immersion during the 3rd hr. [f] Isotonic saline tail vein 3-hr 0.5-ml iv infusion. Whole body 40.0° water bath immersion during the 3rd hr. [g] Forty percent (w/v) dextrose tail vein infusion at rate that maintains 3-hr 400–500-mg % blood glucose level. Whole body 40.0° water bath immersion during the 3rd hr.

RESULTS AND DISCUSSION

All mice (cages 1–12) were subcutaneously transplanted with S-37, given B complex, anesthetized, secured, infused (dextrose or saline), subjected to blood sampling, immersed 1 hr in a water bath (40.0 or 37.0°), and dried. The use of I and/or II was the only variation.

Blood glucose levels of 450–500 mg % were obtained in 60 ± 15 min by infusing approximately 0.2 ml of the 40% dextrose solution. Blood glucose samples obtained after warming the mice remained at or above 400 mg %. Therefore, 1 hr after discontinuing the infusion or 3 hr after establishing the necessary blood glucose level, the desired concentration still existed. To obviate excessive dextrose or volume problems, no more than 0.5 ml was given to any subject.

Treatment of S-37 transplanted virgin female DBA/2J mice with dextrose (cage 2), 1 hr of warming at 40° (cage 3), or 200 mg/kg ip of II (cage 5) was safe (100% treatment survival) and significantly increased longevity. The dextrose–40.0°/1-hr therapy (cage 7) was safe and effectively increased longevity by 40% over the transplanted controls of cage 1. The adjunctive use of I and/or II (cages 8–10) did not enhance this combination. In this study, only mice treated with 5 mg/kg sc of I (cage 4) failed to show a significant increase in lifespan over the controls of cage 1 according to the Student two-tailed t test with 95% confidence limits.

Male mice transplanted with S-37 responded less dramatically to either the tumor (cage 11) or the dextrose–40.0°/1-hr treatment (cage 12) than did virgin females of the same DBA/2J strain (cages 1 and 7). The control males lived significantly longer than the control females; however, the dextrose–1-hr warmed males lived longer than the male controls but not as long as the treated females.

The results of this study are in good general agreement with those of von Ardenne (6). Under different conditions, 1 hr of warming at 40°[2] increased the length of life for S-37 transplanted mice, and 3-hr 400–500 mg % blood glucose levels (3) were even more effective at safely prolonging longevity. A single application of these two procedures together is safe and appears to effect an additive if not a potentiated longevity increase. The results are not improved upon by concurrently administering I and/or II, although II is effective alone. The tumor is less virulent and more susceptible to the "double attack" in males than in females of the DBA/2J strain.

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