Effect of Intraperitoneal versus Intravenous Glucose Administration on Laser Doppler Flow in Murine FSAII Tumors and Normal Skin

J. Kalmus, P. Okunieff, and P. Vaupel

ABSTRACT

The effects of i.p. versus i.v. glucose administration on laser Doppler flow (LDF) were studied in peripheral tissue areas of murine FSAII tumors implanted s.c. in the hind foot dorsum and in normal skin of conscious C3Hf/Sed mice. LDF was monitored prior to and continuously for 90 min following the administration of glucose, galactose, or mannitol at doses of 5 or 10 mg/g. Results showed that i.p. administration of hyperosmolar solutions was followed by a substantial, dose-dependent flow reduction which was indistinguishable for the various agents at equal osmotic load, and similar in tumor tissue and normal skin. Reductions in LDF are, therefore, primarily caused by hypovolemic hemocoagulation following i.p. administration of hyperosmolar sugar solutions. In contrast, i.v. administration of these solutions at 5 mg/g caused an initial flow increase (most probably due to a transient hypervolemic hemodilution), with a return to baseline readings within 5–10 min. At 10 mg/g i.v., a biphasic change in LDF occurred with an initial, temporary increase and a significant decline thereafter with no recovery within the observation period. This drop in LDF most probably is due to a decrease in cardiac output and an increase in viscous resistance to flow. Since comparable changes were observed with all agents and in both tissues investigated, it is concluded that the alterations in flow pattern following injection of hyperosmolar solutions are neither glucose nor tissue specific. Glucose- or tumor-specific effects, if present at all, must be of secondary importance in the animal model chosen.

INTRODUCTION

Over 60 years ago Warburg (1) studied glucose consumption of tumor slices and reported high rates of lactic acid production even under normoxic (aerobic) conditions. From these experiments Warburg and others postulated that utilization of aerobic glycolysis in energy metabolism is a major pathway utilized by malignancies. Subsequent studies showed, however, that these notions were neither characteristic nor unique to malignant tumors (e.g., aerobic glycolysis is also found in the renal medulla, in the retina, in leukocytes, and in other phagocytic cells). Nevertheless, many researchers have sought to take therapeutic advantage of the relative difference in the glucose metabolism of tumors and most normal tissues. Strategies for cancer therapy based on this metabolic pathway were summarized for the first time by Reiss and Hochwald as early as 1932 (2). Administration of high dose glucose to tumor bearing animals, on first principles, would be expected to result in severe tumor lactic acidosis and associated sensitivity to some anticancer agents and hyperthermia (for a recent publication, see Ref. 3). The experimental approaches to test the ability of glucose to induce tumor acidosis has generally been to deliver high doses of hypertonic solutions by i.p. or i.v. routes (4–12). Over the years, several investigators have unequivocally shown that hyperglycemia can decrease blood flow in rodent tumors. Regarding the pathogenetic mechanisms which lead to vascular prestasis or even stasis in malignancies, however, two disparate general mechanisms have been postulated. One assumes that glucose- and/or tumor-specific effects predominate, and, thus, suggests glucose loading is of potential clinical relevance. The other mechanism suggests that nonspecific systemic effects (e.g., hypovolemic hemocoagulation and/or the resulting reduction in cardiac output) are predominantly responsible for the change in tumor flow after glucose loading, a less clinically relevant situation (see Refs. 4–12).

In a preceding paper, we have shown that in s.c. FSAII tumors, i.p. injection of a hyperosmolar glucose or mannitol solution was followed by a dose-dependent hypovolemic hemocoagulation which was associated with a significant dose-dependent inhibition of LDF (5) in superficial tumor regions and in normal skin (6). Changes in LDF due to specific glucose-mediated or glucose-related phenomena probably were of minor importance in the murine tumor system investigated. In the present study we have used an i.v. route for the administration of hypertonic sugar solutions in order to avoid hypovolemic hemocoagulation, which might have masked glucose- or tumor-specific effects in our earlier study. Again, in this series of experiments there is no clear evidence for a paramount role of glucose- or tumor-specific effects on laser Doppler flow in the murine system investigated.

MATERIALS AND METHODS

Animals and Tumors. Experimental animals were 10- to 12-week-old C3Hf/Sed mice derived from our defined flora mouse colony (13). Animals were provided with sterilized animal pellets and acidified and vitamin K-fortified water ad libitum. Early generation isotransplants of a poorly differentiated fibrosarcoma (FSAII) which arose spontaneously in a female C3Hf/Sed mouse were used. Single cell suspensions were prepared by trypsinization and transplanted s.c. into the dorsum of the right hind foot. The tumor grows rapidly with a volume-doubling time of approximately 2.5 days at a volume of 100 to 200 mm³ and is very weakly immunogenic (14). All experiments were performed on conscious mice. Tumor volumes (V) on the day of study were calculated by an ellipsoid approximation using the 3 orthogonal diameters (d) (V = π/6 x d1 x d2 x d3). Tumor volumes ranged from 52 to 135 mm³ [82 ± 11 mm³ (SE)].

Laser Doppler Flowmetry. The LASERFLO Blood Perfusion Monitor 403A (TSI, Inc., St. Paul, MN) was used for this study. This laser Doppler flowmeter provides a stable, reproducible, and noninvasive method for continuous monitoring of tissue microcirculatory function in peripheral tissue areas. LDF is integrated over a hemisphere of approximately 2 mm² (for further details see Ref. 5). Laser Doppler flow signals were obtained from the tumor surface at central locations or from the normal skin of the contralateral foot. The fiberoptic probe was placed above (but not in contact with) the tissue under study using a micromanipulator. Hence, tissue compression was avoided and there was no disturbance of the microcirculation due to fiberoptic probe manipulations.

The microprocessor of this flowmeter computes several variables (RBC flux, RBC velocity, and number of moving RBC) which were
recorded simultaneously on a multichannel chart recorder (type 6514; Linseis, Selb, West Germany). Data were expressed as relative units which represent percentage values of full scale deflection on the instrument meter (5). Absolute blood flow in tumors cannot be quantified by this technique.

Experimental Protocol. After placement on a Styrofoam pad, the mice were immobilized and the tumor-bearing foot was taped to the pad to minimize movement artifacts during LDF measurements. For i.p. administration of glucose, a 22-gauge, 0.75-inch Teflon catheter (Angio-set; Deseret Medical, Sandy, UT) was used. All i.v. administrations were accomplished by tail vein injection. Once the RBC flux reached a constant reading, the agent was administered via bolus injection. As a rule, 0.5 ml of aqueous glucose solutions (25 or 50%) was given over a 2-min period yielding two different i.p. glucose doses (5 and 10 mg/g). Mannitol and galactose were injected either i.v. or i.p. at 5 mg/g (0.5 ml of a 25% solution) in order to separate glucose-mediated from osmotic effects. For control studies, 0.5 ml of a 0.9% NaCl solution (saline) was injected to examine for the effects of i.p. or i.v. injection of isotonic fluid into the mice. Upon administration of the different agents, LDF signals were recorded for 90 min. Following the observation period, the animals were anesthetized and then sacrificed by an intracardiac injection of a KCl solution. All flow data are reported relative to the baseline RBC flux of the individual tumor in the dead animal (RBC = 0%).

Blood Glucose Concentration. In a separate set of experiments glucose was injected i.p. or i.v. using the above-described concentrations and volumes. Blood glucose concentrations were measured spectrophotometrically using a glucose oxidase test kit (Glucose Procedure No. 510; Sigma Diagnostics, St. Louis, MO). Blood samples (50-μl microsampling pipets) were withdrawn from the ophthalmic plexus of tumor-free mice. Blood sugar levels were measured before and 15, 30, 60, and 90 min post i.p. injection, and before and 5, 10, 20, 40, and 90 min after i.v. administration. For each glucose dose, blood glucose concentration was measured in 5 animals.

Modifications of Relevant RBC Parameters after i.p. or i.v. Glucose. Quantitative data on the effect of different glucose doses i.p. or i.v. on relevant RBC parameters were obtained in another series of experiments. Hematocrit values in blood withdrawn from the ophthalmic plexus (in 20-μl capillary tubes) of tumor-free animals were determined before and 15, 30, 60, and 90 min post i.p. injection and 5, 10, 20, 40, and 90 min after i.v. administration of glucose using a standard microhematocrit technique (capillary tube centrifugation). RBC, whole blood hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin content, and mean corpuscular hemoglobin concentration were evaluated using an ELT-80 counter (Ortho Diagnostic Systems Div., Becton Dickinson, Braintree, MA). Using the counter procedure, hematocrit was also calculated and confirmed centrifugation results.

Statistical Analyses. Values given in this study are means ± SE if not otherwise stated. The double tail t test and Scheffe's test were used to determine statistically significant differences.

RESULTS

Blood Glucose Concentrations during Hyperglycemia. The average results obtained from the blood glucose measurements are shown in Fig. 1 (top) (each symbol represents the mean ± SE of 5 animals at a given time). After i.p. glucose injection, the highest blood glucose concentrations measured occurred 15 min postinjection. Blood glucose levels then decreased towards the baseline. No significant changes in blood glucose levels were obtained after bolus injection of saline (0.5 ml i.p. or i.v.).

After i.v. glucose administration, the blood glucose concentration peaked at t = 5–10 min postinjection, again to a dose-dependent value. At comparable doses, peak glucose levels after i.v. administration were significantly higher than after i.p. injection. Blood glucose levels then declined at different rates after i.v. or i.p. loading and reached similar values 30–40 min after injection.

Statistical Analyses. Values given in this study are means ± SE if not otherwise stated. The double tail t test and Scheffe’s test were used to determine statistically significant differences.

Modifications of Relevant RBC Parameters during Hyperglycemia. The average results obtained from hematocrit determinations are shown in Fig. 1 (bottom) (each symbol represents the mean ± SE of 10 animals). Following i.p. glucose injection, there are significant increases in the blood hematocrit values (+30% at 15 min following 10 mg/g), in the number of RBC (+27%), and in the whole blood hemoglobin concentration (+28%). After i.v. glucose injections, there are transient (t = 0–20 min), dose-dependent decreases in the blood hematocrit values (~32% at 5 min following 10 mg/g), in the number of RBC (~28%), and in the whole blood hemoglobin concentration (~28%). All parameters fully normalized by 40 min post-i.v. glucose.

Mean corpuscular volumes, mean corpuscular hemoglobin content, and mean corpuscular hemoglobin concentration did not change significantly after i.v. or i.p. glucose. These results clearly indicate that (a) a dose-dependent hypovolemic hemodilution was present during the total observation period after glucose i.p. and (b) a transient, dose-dependent hypervolemic hemodilution (hypertonic hyperhydration) is attained by
HYPERGLYCEMIA AND LASER DOPPLER FLOW IN MURINE TUMORS

Fig. 2. Laser Doppler flow in murine FSAll tumors (top) and in normal skin (bottom) versus time after i.p. or i.v. glucose loading at a dose of 5 mg/g. Values are means ± SE (Aars).

Modifications in Laser Doppler Flow after i.p. or i.v. Glucose. Whereas no changes in RBC fluxes were observed after i.p. administration of saline (0.5 ml), after i.v. injection of a comparable volume laser Doppler flow tended to increase marginally (value not significant) both in peripheral tumor areas and in normal skin. Glucose at doses of 5 mg/g led to differential changes in LDF when delivered i.p. or i.v. both in tumor and in skin. After i.p. administration of glucose, LDF significantly dropped in both tissues reaching minimum values after ~60 min (see Fig. 2) with no recovery during the observation period. There were no significant differences between the flow decline in skin compared with tumor. Following i.v. administration of glucose, RBC fluxes transiently increased shortly after glucose loading and then returned to baseline levels in both tissues (Fig. 2). These initial rises in LDF following glucose i.v. at 5 mg/g were preferentially due to a substantial increase in mean RBC velocity. As a rule, the drop in LDF after i.p. glucose was caused by both a decrease of the mean velocity of RBCs and, to a lesser extent, a drop in number of moving RBCs. Mannitol or galactose at 5 mg/g either i.p. or i.v. yielded comparable effects on LDF in tumors and in skin, indicating that the changes observed were neither glucose nor tissue specific at the dose chosen (see Table 1).

Glucose at a dose of 10 mg/g i.p. was followed by a substantial flow drop both in peripheral tumor areas and in skin. Nadir flow values were observed after ~60 min in both tissues that did not recover within the observation period (see Fig. 3). In contrast, comparable glucose doses given i.v. initially increased LDF in both tissues for about 10 min. Thereafter, a significant flow drop occurred reaching nadir values 60 min postinjection. Flow inhibition was somewhat less pronounced after i.v. than after i.p. glucose in both tissues. A similar biphasic flow change in both tissues was observed after galactose i.v. at 10 mg/g, again indicating that these changes were neither glucose nor tissue specific (see Table 2).

In all experimental series, pronounced intertumor variability in LDF patterns following i.p. or i.v. administration of the different agents was a common finding. No clearcut correlation was found between the extent of the flow declines and tumor size (within the range investigated) or the magnitude of RBC flux before administration of the agents.

DISCUSSION

Hyperglycemia has been shown to decrease blood flow in many rodent tumor models (3–12). Concerning the postulated pathophysiological mechanisms that might lead to reductions in tumor blood flow, however, there is some disparity when different tumor models, routes of administration, techniques for measuring blood flow, blood glucose levels, and different measuring volumes (e.g., global flow versus regional flow, global flow versus flow in peripheral tissue areas) are considered. In a recent study on murine tumors we have suggested that reductions in laser Doppler flow in peripheral tissue areas following

Table 1  LDF (relative units) in peripheral areas of FSAll tumors versus time before (t = 0 min), and at certain time intervals after (t = 5–90 min) i.p. or i.v. injection of glucose, galactose, or mannitol (dose, 5 mg/g)

<table>
<thead>
<tr>
<th>LDF</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose i.p.</td>
<td>1.0</td>
<td>0.72 ± 0.08*</td>
<td>0.59 ± 0.09*</td>
<td>0.46 ± 0.06*</td>
<td>0.30 ± 0.06*</td>
<td>0.30 ± 0.05*</td>
</tr>
<tr>
<td>Galactose i.p.</td>
<td>1.0</td>
<td>0.76 ± 0.10*</td>
<td>0.61 ± 0.09*</td>
<td>0.50 ± 0.08*</td>
<td>0.42 ± 0.07*</td>
<td>0.40 ± 0.06*</td>
</tr>
<tr>
<td>Mannitol i.p.</td>
<td>1.0</td>
<td>0.78 ± 0.06*</td>
<td>0.59 ± 0.04*</td>
<td>0.53 ± 0.09*</td>
<td>0.64 ± 0.08*</td>
<td>0.61 ± 0.07*</td>
</tr>
<tr>
<td>Glucose i.v.</td>
<td>1.0</td>
<td>1.15 ± 0.06*</td>
<td>1.01 ± 0.07</td>
<td>0.92 ± 0.07</td>
<td>0.97 ± 0.12</td>
<td>1.02 ± 0.10</td>
</tr>
<tr>
<td>Galactose i.v.</td>
<td>1.0</td>
<td>1.25 ± 0.12*</td>
<td>0.99 ± 0.09</td>
<td>0.90 ± 0.04</td>
<td>0.86 ± 0.15</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td>Mannitol i.v.</td>
<td>1.0</td>
<td>1.20 ± 0.15*</td>
<td>1.13 ± 0.15</td>
<td>1.07 ± 0.14</td>
<td>0.85 ± 0.13</td>
<td>0.88 ± 0.15</td>
</tr>
</tbody>
</table>

* P < 0.001.

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Table 2 LDF (relative units) in peripheral areas of FSall tumors versus time before (t = 0 min), and at certain time intervals after (t = 5-90 min) i.p. or i.v. injection of glucose or galactose (dose, 10 mg/g)

<table>
<thead>
<tr>
<th>LDF</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
<th>20 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose i.p.</td>
<td>1.0</td>
<td>0.52 ± 0.05⁹</td>
<td>0.36 ± 0.05⁹</td>
<td>0.28 ± 0.06⁹</td>
<td>0.21 ± 0.03⁹</td>
<td>0.21 ± 0.05⁹</td>
</tr>
<tr>
<td>Galactose i.p.</td>
<td>1.0</td>
<td>0.49 ± 0.07⁹</td>
<td>0.35 ± 0.05⁹</td>
<td>0.24 ± 0.03⁹</td>
<td>0.19 ± 0.03⁹</td>
<td>0.18 ± 0.04⁹</td>
</tr>
<tr>
<td>Glucose i.v.</td>
<td>1.0</td>
<td>1.42 ± 0.27⁹</td>
<td>1.07 ± 0.20</td>
<td>0.71 ± 0.13</td>
<td>0.44 ± 0.07⁹</td>
<td>0.65 ± 0.10⁹</td>
</tr>
<tr>
<td>Galactose i.v.</td>
<td>1.0</td>
<td>1.45 ± 0.45⁹</td>
<td>0.90 ± 0.17</td>
<td>0.61 ± 0.04⁹</td>
<td>0.33 ± 0.03⁹</td>
<td>0.43 ± 0.03⁹</td>
</tr>
</tbody>
</table>

* P < 0.001.
⁺ P < 0.05.

Fig. 3. Laser Doppler flow in murine FSall tumors (top) and in normal skin (bottom) versus time after i.p. or i.v. glucose loading at a dose of 10 mg/g. Values are means ± SE (bars).

i.p. injection of hyperosmolar glucose solutions are predominantly caused by hypovolemic hemococoncentration. Changes in LDF due to specific glucose-mediated or glucose-related phenomena seemed to be of secondary importance in the murine tumor system investigated (3, 5).

In the latter study, laser Doppler flowmetry was used to monitor tumor microcirculatory function in peripheral tumor areas. Although changes in LDF of superficial tissue regions can reflect relative changes of total flow (e.g., Refs. 4 and 15), informations on global flow should be extrapolated only with caution from local measurements at the tumor surface since the latter data are not necessarily sufficient for such conclusions due to substantial flow heterogeneities in malignant tumors (details concerning the validity and major limitations of the laser Doppler flowmetry used to monitor tumor microcirculatory functions have been discussed in Ref. 5).

Examining the effect of glucose given i.v. at a dose of 5 mg/g failed to show any significant decline in LDF in the present study. This is in agreement with our earlier studies on DS-carcinosarcomas growing s.c. (glucose dose, 4.8 mg/g/h; see Ref. 4), or as a tissue-isolated preparation in Sprague-Dawley rats (glucose dose, 1.5–2 mg/g/30 min; see Ref. 16). The transient increase in LDF immediately after i.v. glucose administration most probably is caused by a temporary hypervolemic hemodilution.

Glucose at 10 mg/g i.v. led to a biphasic change in LDF in the murine tumors investigated. After an initial flow increase (again due to a hypervolemic hemodilution), a substantial drop in the RBC flux occurred that did not recover during the 90-min observation period. This flow drop is in agreement with earlier studies describing a shutdown in tumor blood flow after glucose i.v. when given as either bolus injection (6, 17) or as continuous infusion (18–20) in rabbits or rats.

Using conscious, unrestrained rats, DiPette et al. (17) have shown that the flow drop observed in tumors and normal tissues following i.v. administration of glucose at a dose of 6 mg/g is caused by a substantial decrease in cardiac output without changing blood pressure or heart rate. This pattern of parameter changes is caused by an increase in total peripheral resistance and a concomitant decrease in stroke volume (17). These authors also described a larger flow drop following i.p. injection than after i.v. administration (21). This observation in rats is in accordance with the flow changes described in this study.

Alterations in RBC flux reported in the present study are comparable in peripheral tumor areas and in normal skin. Furthermore, glucose, galactose and mannitol given i.v. at comparable doses yielded similar changes in tumor and normal skin. This is in line with observations described by DiPette et al. (22) suggesting that the effect of galactose on blood flow rate in rats is similar to that produced by glucose. These findings are also consistent with those of Urano et al. (23) in which glucose and mannitol induced similar increases in radiobiologically hypoxic cell fraction and enhancement of the effects of hyperthermia on tumors and of hyperthermic toxicity to the mouse foot (24). From this, we conclude that the flow variations described are neither glucose nor tissue specific in the mouse model used. Changes in RBC flux after glucose i.v. at 10 mg/g most probably are caused by a decrease in cardiac output (17) and an increase in viscous resistance to flow (and thus to a rise in total peripheral resistance) which again seems not to be glucose specific. Since similar changes were also observed fol-
flowing i.v. galactose, this effect was most probably caused by the hyperosmolar solutions used, which mandatorily led to a forced osmotic diuresis and profoundly endangered the animals. In the mouse tumor model used, the i.v. injection of the different agents led to a transient increase in LDF within peripheral tumor regions and in normal skin. This is in contrast to data reported by Ward-Hartley and Jain (6). In the latter study which emphasizes a glucose-induced increase in blood viscosity (that was partially due to an increase in RBC rigidity) as a major effect responsible for the reduction in tumor blood flow, neither saline nor glucose nor galactose i.v. could increase the flow rate even temporarily in mature granulation tissue or tumors in rabbits. Whether this differential flow behavior is species, tumor, or methodology related must be clarified in future studies. However, it should be mentioned that in the present mouse study i.v. fluid loading was 2.5 times higher (20 versus 8 µl/g) than in the rabbit study. This difference could partially account for the discrepancies in the experimental results.

REFERENCES

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