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Dose-effect Relationships for Glucose-induced Tumour Acidification and its Erythrocyte Flux

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Preclinical investigations were performed with glucose administration in WAG/Rij rats carrying the rhabdomyosarcoma BA1112 in two sites per animal: one in the subcutis of the flank (for pH measurements in the tumour tissue) and one in the transparent "sandwich" chamber for measuring the erythrocyte flux in the tumour tissue as an indication for changes in tumour blood flow. A glucose solution (20%) was slowly infused intravenously in a range of dose levels, similar to those reported for inducing long-term hyperglycaemia in man. The eventual aim of such investigations is to sensitise tumours for hyperthermic treatment. This approach is not new, but the present experiments were performed with the aim to explore the level of the minimal amount of glucose which would nonetheless yield a likely therapeutic effect. Endpoints in this study were the blood glucose level and pH and the relative erythrocyte flux in the tumour tissue. Obviously, as one would expect, many significant changes in the various parameters were found as a response to administration of glucose. However, the changes in the blood glucose level, the induced decrease in tumour pH and the influence of the tumour volume did not show a well-defined relationship which was reliable enough to predict the exact influence of the various parameters on the magnitude of the desired changes in individual animals and/or tumours. This was probably caused by interfering differences in physiological feedback mechanisms. Nonetheless, the data indicate that the optimal effect was not obtained with the highest treatment level, but with moderate doses of glucose, i.e. 2.4–3 g.kg/h which induced a satisfactory tumour acidification of 0.25 pH units. This may turn out to a clinically useful pH drop for enhancing the cytotoxic effect of hyperthermia. The erythrocyte flux through the tumour tissue does not appear to be influenced to a sizeable extent by such a treatment.
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INTRODUCTION

THE OBSERVATION that the interstitial pH of human and experimental tumours is low, and can be decreased by the application of glucose, has been known for about four decades [1–5]. This may be exploited for therapeutic benefit [6–14]. In addition, it has been shown that hyperthermia can decrease the intratumoral pH in experimental tumours [15–17], provided that the treatment temperature exceeds 42°C [18]. Also, in human tumours, a hyperthermia-induced decrease in tumour pH has sometimes been observed [19, 20].

The glucose content of experimental tumours and of the interstitial fluid of tumours is generally low [21, 22], depending on the size. The glucose content of very small experimental tumours is rather high (P. Vaupel, Institute of Physiology and Pathophysiology, University of Mainz). The cause for a low interstitial pH in tumours is generally thought to be the insufficient supply of oxygen, resulting in an anaerobic metabolism, with an impaired egress of the acidic metabolites through the sluggish tumour blood flow. In this concept, the oxygen in this tissue is depleted earlier than the glucose. If glucose is administered systemically, i.e. via an intravenous or intraperitoneal route, the supply of glucose in the tumour tissue is increased, however without an increase in the removal of the acidic metabolites. This results in acidification of the tumour tissue [23]. It should, however, be realised that a high single dose of glucose 5–6 g/kg, as used in most experimental investigations, also elicits other physiological disturbances, such as a decrease in tumour blood flow [16], which is the likely result of a decreased cardiac output [24]. Moreover, under hyperglycaemic conditions the potassium level of the blood shows alterations which may induce physiological disturbances [25, 26]. Recently, it has been unequivocally demonstrated that after the customary high intraperitoneal doses of glucose, a severe fluid shift occurs, which results in haemoconcentration [27].

This results in a clinically unacceptable and unrealistic physiological condition, obscuring any real gain that might be achieved by combining elevated glucose levels with hyperthermic treatment. This problem formed the basis of the present investigations, i.e. we wanted to answer the question whether moderate dosages of glucose would result in changes in the tumour interstitial pH to an extent that such a treatment would be worthwhile. In addition, the question was addressed whether such moderate dosages of glucose would influence erythrocyte flux in the tumour to any sizable extent.

MATERIALS AND METHODS

The tumour used for these investigations was the undifferentiated rhabdomyosarcoma BA1112, which is isogenous in the WAG/Rij strain of rats. This tumour was transplanted first in the flank of 14-week old female rats, already carrying the transparent "sandwich" system [28] in the skin of their back. When the tumour in the flank had reached a diameter of about 4 mm, a tiny piece of tumour was transplanted in the sandwich system. In this way, about 7 days later a sandwich tumour of about 3 mm in diameter was available, as well as an 8–12 mm tumour in the subcutis of the flank.

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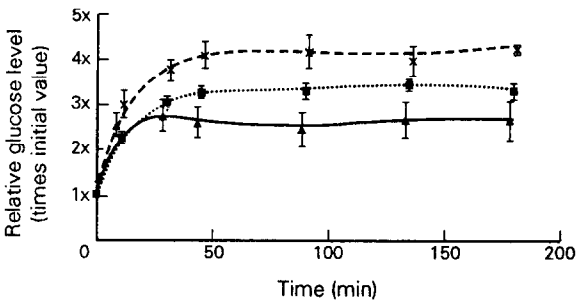

Fig. 1. Relationship between the dose-rate of glucose and the relative elevation of the blood glucose level in female WAG/Rij rats, average weight 165 g. Bars indicate S.E. Infusion rate of glucose: —— ≤ 2.4, · · · · 2.4–3, — — — ≥ 3 g/kg/h.

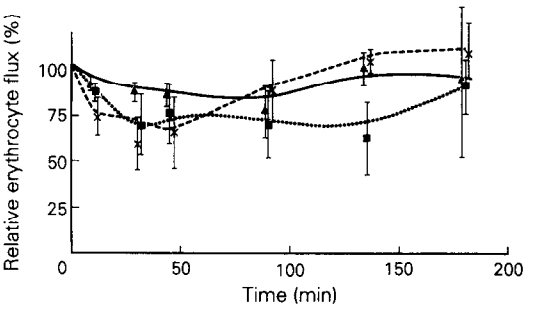

Fig. 3. The relative erythrocyte flux in rhabdomyosarcoma BA1112, in the same animals as in Figs 1 and 2. Infusion rate of glucose: —— ≤ 2.4, · · · · 2.4–3, — — — ≥ 3 g/kg/h.

The animals were anaesthetised with 50 mg/kg pentobarbitone intraperitoneally, and positioned in a special holder [28] under the stereomicroscope. This holder also provided means to selectively heat the skin flap with the sandwich tumour in a very accurate way. An additional optical set-up allows the measurement of the velocity of the erythrocytes in selected capillaries [29]. Before positioning the animal, a cannula was fitted in the jugular vein. This cannula is for administering of the glucose (20%) via an adjustable infusion pump. Primary to the continuous intravenous infusion a priming dose of 0.5 g/kg was given via this system.

In addition, a glass pH electrode of 2.1 mm diameter (Philips C 902S, [3]) was inserted into the tumour in the flank.

The glucose content of the blood was measured with a Reflolux II glucose meter (Boehringer, Mannheim) from samples taken at the tail tip at time zero, and 15, 30, 45, 90, 145 and 180 min after the start of the continuous glucose infusion. At the same time points the relative velocity of the erythrocytes in the tumour capillaries and tumour tissue pH were determined. The dose rate to be given was somewhat dependent on the weight of the animals, as the glucose infusion pump could only be adjusted in a series of discrete steps. There were 21 animals and three dose rates. With the aforementioned seven time points, the number of observation data was 147.

RESULTS

As expected, the blood glucose level rose as a result of the intravenous administration of glucose in a dose-dependent manner (Fig. 1). For the purpose of clarity the data obtained with the dose levels have been divided into three groups. This was justified because the individual dose rates in these three groups yielded comparatively similar changes in blood glucose levels per group. In Fig. 1, the relative changes in blood glucose levels are depicted. The mean initial blood glucose levels (mg %) of the three groups were 73 (S.E. 2), 72 (1) and 72 (3) in the order of increasing glucose dose rate.

The tumour pH changes decreased with increasing glucose level to a certain extent (Fig. 2). However, the intermediate dose-rate of glucose, i.e. between 2.4–3 g/kg/h appeared to be slightly more effective in decreasing the tumour pH than the highest dose rate. The mean initial pH-values of the tumours of the three groups were 7.11 (S.E. 0.03), 7.21 (0.02) and 7.08 (0.06) in the order of increasing glucose dose rate. One-way analysis of variance showed that these values were not significantly different ($P < 0.01$). The changes in erythrocyte velocity are shown in Fig. 3. It appears that, especially during the administration of the highest dose rate of glucose (≥ 3 g/kg/h), there is an initial decline, which is followed by a recovery during the continuation of the infusion. The intermediate dose rate of 2.4–3 g/kg/h results in a modest reduction in erythrocyte velocity to about 75% of the initial value.

The interrelationships between the various parameters were examined with a Spearman rank correlation table, taking all available data into account (Table 1). This table also shows that the relative tumour erythrocyte flux is not influenced by the glucose treatment to a significant extent. There appears to be an incidental correlation between tumour volume and dose rate of glucose, which can not be regarded as a causal relationship in view of the set-up of the experiments. The pH drop in the tumour is more strongly correlated with the cumulative dose of

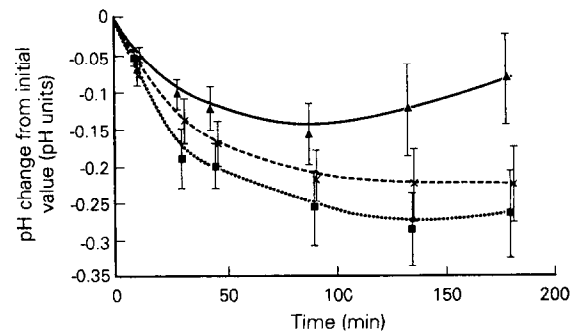

Fig. 2. Induced decrease in tumour pH (rhabdomyosarcoma BA1112), in the same animals as in Fig. 1. Infusion rate of glucose: —— ≤ 2.4, · · · · 2.4–3, — — — ≥ 3 g/kg/h.

Table 1. Spearman rank correlation coefficients (R_{Sp})

	Dose rate	Cumulative dose	Blood glucose	Tumour volume	pH drop
Dose rate of glucose	1				
Cumulative dose of glucose	0.23	1			
Blood glucose level	0.42	0.67	1		
Tumour volume	−0.42	0.01	−0.14	1	
pH drop in tumour	−0.16	−0.61	−0.45	−0.02	1
Relative tumour RBC flux	0.08	−0.06	−0.11	0.14	0.15

$n = 147$.
Two-tailed probabilities were: $2P \leq 0.05$ for $r_{Sp} \geq 0.16$; $2P \leq 0.01$ for $r_{Sp} \geq 0.23$.

glucose than with the blood glucose level. Tumour volume appears to have no influence on the glucose-induced pH drop.

DISCUSSION

The results of these investigations show that a moderate amount of glucose, administered intravenously at a dose rate of 2.4–3 g/kg/h induced a satisfactory drop in the pH of the tumour tissue of about 0.25 pH units. It should be mentioned here that a 2 mm diameter pH electrode, measures the average pH of destroyed cells and extracellular fluid. Higher dose rates than 2.4–3 g/kg/h do not apparently result in increasing the acidifying effect. The nadir of the pH drop seems to be reached after about 90–130 minutes, or for practical purposes, after about 1 1/2 hours.

The pH drop obtained in the present experiments was 0.25 pH units. This is somewhat less than the 0.5 unit which emerges in the simplified diagram published by Von Ardenne and Reitnauer [30], but our value is similar to that published for RIF tumours by Tobari *et al.* [31]. Jähde and Rajewsky [23] induced a tumour pH drop of about 0.65, for which a 10-fold increase in serum glucose was required.

The pH drop induced in the present experiments was slightly less than that obtained by Osinsky [13], who probably used a higher dose rate of glucose. This pH drop went from 6.73 to as low as 5.2–5.4. These experiments do not yet shed any light on the intriguing observation by Ward-Hartley and Jain [32] that a difference in effect apparently exists between glucose and galactose, given as a bolus of 6 g/kg on the blood flow of the rabbit VX_2 carcinoma and the normal, mature granulation tissue.

It is interesting to note here that Vaupel *et al.* [33] with a continuous infusion rate of 4.8 g/kg/h observed a stronger effect on the decrease of tumour blood flux, as measured with the laser-doppler method, and that this effect was more pronounced during 42°C heating than at 44°C. Under these conditions the response of the erythrocyte flux seems therefore stronger than in the present experiments, shown in Fig. 3.

The elevated levels of serum glucose induced in the present experiments are slightly higher than the ones induced in volunteers for different purposes [34]. The long-term high level (400 mg%) hyperglycaemia, as used by Lippman and Graichen [25] requires monitoring and correction of electrolytes. A very recent publication by Krag *et al.* [35], however, does not mention this as a particular problem. On the other hand, oral administration [36] was apparently also well tolerated, but the change in tumour pH in these clinical investigations was modest, and not very predictable. The present experiments indicate that it may be worthwhile to investigate clinically the effect of a 2–3 fold increase in blood glucose level by starting the glucose infusion about 1–1 1/2 hours before the hyperthermic treatment. Preliminary analysis of an ongoing series of rat experiments seems to indicate a glucose induced gain in tumour control dose (TCD_{50}) of about 10 Gy, when radiotherapy is combined with hyperthermia.

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Interaction between Hormone-dependent and Hormone-independent Human Breast Cancer Cells

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We developed two different models based on *in vitro* co-culture of hormone-dependent and hormone-independent cell lines to simulate the cell population heterogeneity of human breast cancer tumours. Oestrogen-dependent (MCF-7, ZR 75.1) and oestrogen-independent cell lines (MDAMB-231 BT-20) were grown under serum-free conditions. Co-culture of hormone-dependent and hormone-independent cell lines resulted in an increased cell yield compared to single cell cultures carried out at the same seeding ratios. Such an increase was not affected by addition of oestradiol and single growth factors (EGF, bFGF and IGF-I). These results allow us to conclude that in a heterogeneous cell population like human breast tumours, interaction between hormone-dependent and hormone-independent cell lines may result in a complex regulation of cell growth.
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INTRODUCTION

DURING THE last decade many cell lines have been characterised and used to investigate the growth modality of breast cancer [1]. On the basis of receptor status and of their dependence on oestrogens, cell lines have been subdivided into endocrine-dependent and endocrine-independent and have been separately investigated. However, human breast tumours are a heterogeneous mixture of different cell populations, which are characterised by variable degrees of hormone dependence [2], so that the growth of a tumour may be the result of the individual properties of cell subpopulations and of their interaction. Endocrine-dependent cells are oestrogen-receptor positive, and their proliferation rate is increased by the addition of oestradiol [3, 4]. They do not constitutively produce growth factors (GFs), but after administration of oestradiol (E_2) an induction of GFs has been observed [5, 6]. Conversely, endocrine-independent cell lines produce GFs constitutively and do not increase their proliferation rate as a response to E_2 [7]. This would imply that in the absence of hormones, oestrogen receptor positive (ER+) cells can be induced to proliferate by GFs released by oestrogen receptor negative (ER−) cells present in the heterogeneous tumour cell population. Indeed, it is well known that GFs such as epidermal (EGF) basic fibroblast (bFGF) and insulin-like GF (IGF-I) can stimulate ER+ cells to proliferate also in the absence of E_2 [8].

To improve knowledge on this biological aspect, we studied the interaction between ER− and ER+ breast cancer cell lines under several culture conditions. The study was performed by using mixed cell cultures to allow cell contact or transwell systems to limit cell interaction to diffusible substances.

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