Reports

Potentiation of 5-Fluorouracil Inhibition of Flexner-Jobling Carcinoma by Glucose

Abstract. *A significant potentiation of the antitumor action of 5-fluorouracil in rats bearing the Flexner-Jobling carcinoma is produced by the intraperitoneal administration of large doses of glucose.*

The 5-fluorouracil (5-FU) and 6-azauracil (6-AzaU) analogues of uridine diphosphoglucose (UDP-glucose) serve as substrates for UDP-glucose dehydrogenase (*1, 2*). Moreover, striking effects on the Michaelis constants (K_m) of the unnatural substrates are observed with change of *p*H. The K_m values for 6-azaUDP-glucose and FUDP-glucose are about 40- and 13-fold lower, respectively, at *p*H 7.0 than they are at *p*H 8.7. The K_m for UDP-glucose shows about a threefold decrease in this *p*H range. At *p*H 7.0 and below, the K_m values of all three substrates are of a similar order of magnitude. Essentially similar observations were made with a second enzyme, UDP-glucuronyl transferase and the analogue substrates, FUDP-glucuronic acid and 6-azaUDP-glucuronic acid (*3*).

These findings are explained by the marked differences in the pK_a values of the three substrates. The pK_a of UDP-glucose is 9.5 (*2*); of FUDP-glucose, 7.7 (*4*); and of 6-azaUDP-glucose, 6.8 (*5*). Thus the ionic state of the pyrimidine moiety has a determinative effect on the binding of substrate to enzyme and it is the undissociated form of the substrate that is the effective species for interaction with the enzyme. Indeed the K_m values of the three substrates expressed in terms of the concentrations of the un-ionized substrate in solution are all similar (*2*).

That these observations may have wider significance is suggested by several considerations. Similar *p*H effects with pyrimidine analogue substrates were recently described (*6*). Furthermore the pK_a values due to ionization of the pyrimidine rings of all nucleosides and nucleotides of a particular base are alike, and at a given *p*H approximately the same ratio of un-ionized to ionized species will exist with the ribose derivatives of a particular base analogue regardless of the enzymic reaction studied. Also it should be recognized that the degree of ionization may have a marked effect upon the transport of a drug into cells and that in many instances anions diffuse through cell membranes only with difficulty (*7*).

Since Warburg's classic discovery (*8*) it has been known that the *p*H of many tumors may be below that of normal tissues as a result of high aerobic glycolytic capacity and the accumulation of lactate. The aforementioned observations which indicate that the antitumor pyrimidine analogues, 5-FU and 6-AzaU, are more effective in a more acidic environment suggest that some of the differential growth inhibitions of these analogues may be due, at least in part, to the greater acidity of many tumor tissues. If this is so, measures which lower still further the *p*H of tumors might also produce a significant potentiation of the carcinostatic action of the antipyrimidines, 5-FU and 6-AzaU, and of other agents whose pK_a values are in the physiological range. The published reports (*9, 10*) that intraperitoneal administration of glucose can produce a prolonged decrease to levels of 6.3 to 6.5 in the *p*H of certain tumors (including the Flexner-Jobling carcinoma in rats) offered a relatively simple means of testing this possibility (*11*).

Female Holtzman rats (110 to 120 g) were implanted in the right axillary region with Flexner-Jobling carcinoma (*12*) by the usual trocar technique. Nine days after inoculation the experimental groupings were formed by sorting the animals so that the tumor-size distribution within each group was comparable. Animals which were not susceptible and those bearing unusually large or small tumors were rejected; the rejects numbered 10 to 20 percent of those inoculated. Animal weights and tumor sizes were determined during the 7 days of treatment; on the 8th day the animals were killed and the tumors were weighed to the nearest 10 mg.

The animals were given glucose (5 g/kg of body weight) or isotonic saline intraperitoneally 2 hours prior to treatment and then 5-FU (*13*) of the appropriate dosage plus either glucose (2.5 g/kg) or isotonic saline. The volume administered was 2 ml in each case. When galactose was substituted for glucose the same dose regimen was followed.

In Table 1 are presented the results of four separate experiments, each of which demonstrates a significant potentiation of the antitumor action of 5-FU by the administration of glucose. Definite tumor inhibition was observed when glucose was included in the treatment even at the low dose of 10 mg of 5-FU per kilogram ($P < 0.01$). This dose when administered with saline did not give significant inhibition of control tumors ($P > 0.2$). In two experiments the mean tumor size with glucose treatment alone was somewhat smaller than that of the saline controls. To eliminate a possible slight effect of the glucose treatment on tumor size, the data for

Table 1. The potentiation of the antitumor action of 5-FU by glucose. Level of significance of difference in tumor weights was calculated by the *t* test.

Dose of 5-FU (mg/kg)	Average weight of tumor in grams		
	Saline	Glucose	*P**
	Experiment 1 (8 rats per group)		
0	4.65 ± 1.23	4.30 ± 1.23	> 0.5
	P† > 0.2	*P*† ≦ 0.01	
10	3.63 ± 0.93	2.78 ± 0.69	≦ 0.05
	Experiment 2 (12 rats per group)		
0	2.30 ± 0.73	1.82 ± 0.77	> 0.1
	P† > 0.5	*P*† < 0.01	
10	2.11 ± 0.98	1.05 ± 0.57	< 0.01
	Experiment 3 (10 rats per group)		
0	5.63 ± 1.35	4.44 ± 0.77	< 0.02
	P† > 0.5	*P*† < 0.01	
10	5.22 ± 1.35	3.44 ± 0.69	< 0.001
15	4.84 ± 0.54	2.90 ± 0.66	< 0.001
30	3.11 ± 0.52	1.82 ± 0.64	< 0.001
	Experiment 4 (6 rats per group)		
0	5.93 ± 1.65	5.48 ± 1.42	> 0.5
	P† > 0.4	*P*† < 0.01	
10	5.33 ± 1.20	3.27 ± 1.17	< 0.001
20	4.10 ± 1.25	2.37 ± 0.85	< 0.02
30	2.91 ± 1.56	2.32 ± 0.68	> 0.4
40	2.16 ± 1.36	1.33 ± 0.55	> 0.2

* Comparison between tumor weights at given doses of 5-FU with and without supplementary glucose. † Comparison between tumor weights at 5-FU dose of 10 mg/kg and the respective saline or glucose control.

each dosage of 5-FU were compared with the appropriate saline or glucose control value and plotted log-dose against probit percent. The regression lines obtained indicated that 50-percent inhibition of tumor growth is caused by a dose of about 32 mg of 5-FU per kilogram when given with saline and by a dose of about 18 mg with concurrent glucose treatment. Since the pK_a values of 5-FU nucleosides and nucleotides are 7.6 to 7.7, at the physiological *p*H of 7.4 these compounds are about 60 percent undissociated; at *p*H 6.3 the level reached in tumors after glucose treatment (*9*, *10*), the 5-FU ribose derivatives would be 95 percent un-ionized. Thus the percentage increase in the antitumor activity may be related to the increase in the percentage of un-ionized species of nucleotide that is present.

An experiment was performed to test whether 5-FU antitumor action can be enhanced by galactose administration (in the same regimen as for glucose in Table 1). Treatment with this hexose was tested because, in contrast to the effect of glucose treatment, it does not cause lactate accumulation and a lowered *p*H in the Flexner-Jobling carcinoma (*9*). The tumor sizes after 7 days of treatment were (in grams wet weight ± S.D.): saline alone, 6.5 ± 1.5; galactose alone, 6.5 ± 1.5; saline + 5-FU (10 mg/kg), 6.6 ± 1.6; galactose + 5-FU (10 mg/kg), 6.5 ± 1.9; glucose + 5-FU (10 mg/kg), 4.8 ± 0.9. Thus galactose had no measurable effect on the tumor response to 5-FU while the expected response to concurrent glucose therapy was observed ($0.01 > P > 0.001$).

The question arises whether glucose treatment increases the toxicity of 5-FU as well as its antitumor effect; that is, does the therapeutic index remain unchanged? Present indications are that the toxicity is not significantly affected in the rat by added glucose treatment. At the various doses (Table 1), the average weight change was comparable in the saline and glucose-treated group pairs. Fatalities were observed only at 40 mg of 5-FU per kilogram, where two animals of six in the saline group died and one of six in the glucose group died. Groups of normal rats were administered 5-FU in seven daily doses of 40, 60, 80, and 100 mg per kilogram of body weight with concurrent saline or glucose treatment. All animals that received 60 mg of 5-FU or more per kilogram of body weight per day died within the 7 days of the experiment and those given 40 mg died within 3 days after the end of the treatment. The average times of death at the various 5-FU doses were similar in the saline and glucose-treated group pairs.

The major site of action of 5-FU is the enzyme thymidylate synthetase (*14*), which is profoundly inhibited by 5-fluoro-2′-deoxyuridine-5′-monophosphate. Since this analogue nucleotide is formed from 5-FU in a "lethal synthesis" in which at least four different enzymes participate, *p*H alterations might affect the reaction at thymidylate synthetase, or one or more of the enzymes in the pathway of 5-fluoro-2′-deoxyuridine-5′-monophosphate synthesis. Although enzyme studies showing the analogue nucleotides to be more effective in an acidic environment led to the present experiments, it may not be assumed that glucose potentiation is occurring at the enzyme level. Transport of 5-FU into the tumor cell may be enhanced by the lowered *p*H. Future studies will be needed to determine which, if any, of these explanations is valid.

The pK_a values of other antitumor compounds, such as 8-azaguanine, 6-azauracil, 6-thioguanine, and 6-mercaptopurine and their nucleosides and nucleotides are significantly below those of their respective natural congeners (*15*), but it is not known whether the antitumor action of these compounds may also be enhanced in an acidic tumor environment. Perhaps the therapeutic index can be improved still further by decreasing the toxicity to normal tissues by alkalinization through bicarbonate administration or hyperventilation (*16*).

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11. In preliminary studies the intraperitoneal administration of 5 g glucose per kilogram of body weight in mice caused a high mortality rate, thus rat tumors seemed more suitable for study than mouse tumors.
12. The line of Flexner-Jobling carcinoma used is that carried in the Wisconsin Alumni Research Foundation laboratories.
13. The 5-fluorouracil was a gift of Dr. James Price.
14. L. Bosch, E. Harbers, C. Heidelberger, *Cancer Res.* **16**, 335 (1958).
15. Reported pK_a values are: 8-azaguanosine, 8.5 (R. M. Bock, private communication); 6-azauridine, 6.8 (*5*); 6-thioguanosine, 8.3 (*4*); 6-mercaptopurine riboside, 7.8 (*4*).
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"Microsome" Fraction of Brain: Structural Changes Induced by Ascorbic Acid

Abstract. *The usual vesicular configuration of membrane fragments in the brain "microsome" fraction is radically altered by treatment with ascorbic acid and adenosine monophosphate. Light-scattering measurements and electron micrographs show that the treated membranes assume predominantly planar forms. This change in structure appears to be contingent upon a continued transport of electrons from ascorbic acid.*

Although the ascorbic acid content of neurons in certain parts of the brain such as hypothalmus is remarkably high (*1*), the nature of its role in neural function is completely unknown. Ascorbic acid was found to induce gross structural changes in vitro in the membranous component of the so-called "microsome" fraction of rat brain. Although these structural changes are associated with an oxidation of ascorbic acid, no other potential electron donor, for example, glucose, tricarboxylic acid cyclic intermediates, glutathione, or hydrosulfite, could be substituted for ascorbic acid or D-isoascorbic acid in producing this effect.

This investigation actually began as an attempt to duplicate with microsomes from the brain the finding that ascorbic acid, in the presence of microsomes from several non-neural tissues such as adrenal, liver, and kidney, can activate the oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$) (*2*). However, there was an interference with the optical measurement of this oxidation because of an unexpected change in light scattering in brain microsome fractions that had been treated with ascorbic acid.