Propranolol Hydrochloride Enhancement of Tumor Perfusion and Uptake of Gallium-67 in a Mouse Sarcoma

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The effect of propranolol hydrochloride on the blood perfusion of a mouse sarcoma and other tissues has been studied using ^{86}Rb. The maximum increase in relative tumor perfusion (2x controls) occurred 15 min after an i.v. administration of 10 mg per kg propranolol hydrochloride. To study the effect of this drug on the uptake of ^{67}Ga, it was injected at a concentration of 10 mg/kg 10 min before administering 3 μCi (110 kBq) [^{67}Ga]citrate. Tissue uptakes were measured 4 hr later. The tumor: blood ratio increased from 1.16 ± 0.17 to 3.41 ± 2.27 (s.d.) and tumor: liver ratio increased from 2.39 ± 0.30 to 7.13 ± 3.52 (s.d.). The results showed that propranolol hydrochloride can improve the relative tumor blood flow and radiopharmaceutical concentration in an animal model. It is hoped that this and other agents will yield similar results in the human situation.

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The ability to detect small tumors using tumor localizing agents would be improved by increasing the tumor to background ratio. Attempts have been made to increase the relative tumor concentration of gallium-67 (^{67}Ga) using chelating agents to remove free gallium from background tissues (*1*). This communication describes a technique to improve tumor: background ratios by employing a vasoactive agent to increase the perfusion of tumor relative to other tissues.

In previous studies it has been shown that the anesthetic agent pentobarbitone sodium increased the relative tumor perfusion in experimental tumor in mice through a decrease in blood flow to muscle (*2*). This was thought to be because of a decrease in cardiac output, with compensatory sympathetic vasoconstriction taking place in an effort to maintain the blood pressure. A similar decrease in cardiac output with vasoconstriction is obtained by using propranolol hydrochloride (propranolol HCl) which blocks the beta-adrenoreceptors producing a decrease in heart rate and a lowering of blood pressure.

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It is hoped that this drug, which is in routine clinical use, could be used to improve human tumor perfusion, enhancing the uptake of diagnostic radiopharmaceuticals and the tumor concentration of chemotherapeutic agents.

MATERIALS AND METHODS

The tumor studied was a transplanted mouse sarcoma PM implanted subcutaneously in BALB/c mice, 8–10 wk old, weighing 20–30 g, usually close to the mean of 25 g body weight. Radioactive isotope distributions were normalized to a standard body weight of 25 g.

The tumor arose spontaneously in a BALB/c mouse in these laboratories (*3*) and was passaged by implanting 1 mm^3 pieces subcutaneously in the flanks of mice. Histologic examination showed an undifferentiated sarcoma, with no smooth muscle coats in the blood vessels. The tumors were used 2–3 wk after implantation, when 0.5–1 g in size. Only viable tissue was used; any necrotic centers, which occasionally appeared in the tumors, were discarded.

The blood flow was measured using the rubidium-86 (^{86}Rb) method described by Sapirstein (*4*). All injections were made intravenously through a cannula in-

TABLE 1
Effect of Propranolol Hydrochloride on Perfusion in Mouse Sarcoma (PM)

Time between drug admin. and perfusion determination (min)	Tumor perfusion as mean uptake ^{86}Rb/g tumor ± s.e.m.	
	Saline Control	Propranolol 10 mg/kg
1	2.75 ± 0.17* (14)	3.64 ± 0.11 (4)
5	3.05 ± 0.23 (17)	5.24 ± 0.50† (4)
15	3.07 ± 0.32 (8)	6.02 ± 0.33† (8)
30	2.53 ± 0.31 (5)	2.52 ± 0.11 (5)

* Number in brackets refers to number of mice used.
† $p < 0.001$ by Student's t-test, compared with control values.

serted in the tail vein of conscious mice. No anesthesia or application of heat was used. If necessary, a tourniquet was applied to cause dilation of the vein while inserting the cannula. The drug and ^{86}RbCl (3μCi, 110 kBq) were each injected in a volume of 0.1 ml and were flushed in with 0.1 ml saline. The mice were killed 1 min after the ^{86}Rb injection by injecting ~0.1 ml of saturated potassium chloride solution by way of the cannula. Preliminary experiments using saline injections showed that the tissue distribution of ^{86}Rb was not affected by the prior injection of up to 0.6 ml saline.

Tissue samples were placed in small glass tubes, which were inserted into larger glass test-tubes. The 1-MeV gamma irradiation from ^{86}Rb was counted in an automatic scintillation counter. The double thickness of glass absorbed the 1.77 MeV beta particles associated with the decay of ^{86}Rb. Tissue concentration was calculated as % injected dose per g.

The uptake in each tissue, expressed as % dose/g, represents the fractional distribution of cardiac output. On average the fraction of cardiac output reaching the tumor in control mice injected with a volume of saline equivalent to the volume of the injection containing the beta-blocker was 2.8%.

Propranolol hydrochloride was injected intravenously in two concentrations, namely 5 mg/kg and 10 mg/kg. Blood perfusion in the tumor and other tissues were determined by injecting ^{86}Rb at 1, 5, 15, and 30 min following administration of the vasoactive agent. Propranolol HCl at a concentration of 10 mg/kg produced the maximum changes in perfusion at 15 min following its administration, resulting in a near doubling of the percentage fractional distribution of the cardiac output (percentage FDCO/g) to the tumor.

To assess the effect of the increase in relative tumor perfusion on the uptake of ^{67}Ga, studies were made using propranolol HCl 5 mg/kg and 10 mg/kg injected intravenously 10 min before an i.v. injection of 3 μCi (110 kBq) of [^{67}Ga]citrate. Tissue uptakes of ^{67}Ga at 4 hr following the administration of the radioactive isotope were measured in a well counter.

RESULTS

The effect of propranolol HCl on the sarcoma-bearing mice is seen in Table 1. The tumor perfusion is indicated by the mean fractional uptake of ^{86}Rb per g of tumor. The results show that propranolol HCl in a concentration of 10 mg/kg increases the fractional distribution of cardiac output per g of tumor, from 3.07 ± 0.32% to 6.02 ± 0.33% (s.e.m.). This is significant at $p < 0.001$ by Student's t-test.

Table 2 shows the effect of propranolol HCl on the uptake of ^{67}Ga in the PM sarcoma. It can be seen that propranolol HCl at a dose of 10 mg/kg, injected 10 min before administering ^{67}Ga intravenously, decreases the accumulation of ^{67}Ga in the spleen, liver, and kidneys, and reduces the level in the vascular compartment while the tumor uptake is unchanged. The relative tumor to blood ratio increased from 1.16 ± 0.17 (s.d.) to 3.41 ± 2.27 (s.d.). A similar improvement was found in the case of the tumor to liver ratio which increased from 2.39 ± 0.30 (s.d.) to 7.13 ± 3.52 (s.d.). The mean uptake per g of muscle stayed relatively constant at both concentrations of propranolol HCl.

DISCUSSION

A technique which would consistently improve the uptake of radiopharmaceuticals or chemotherapeutic

TABLE 2
Effect of Propranolol Hydrochloride on ^{67}Ga Uptake in Mouse Sarcoma*

Item	Gallium-67 accumulation as mean % injected dose/g tissue ± s.d.		
	Controls (n = 3)‡	Propranolol 5 mg/kg 10 min pre-gallium (n = 4)	Propranolol 10 mg/kg 10 min pre-gallium (n = 4)
Blood	8.30 ± 0.64	6.70 ± 1.12	3.20 ± 1.30[b]
Tumor	9.55 ± 0.82	8.34 ± 1.80	8.12 ± 1.12
Spleen	2.46 ± 0.15	1.77 ± 0.26[b]	0.86 ± 0.26[a§]
Liver	4.00 ± 0.21	2.60 ± 0.26[a]	1.33 ± 0.39[a]
Kidneys	4.76 ± 0.22	3.57 ± 0.61[c]	2.67 ± 0.35[a]
Muscle	1.07 ± 0.55	1.28 ± 0.08	1.36 ± 0.14
Tumor/blood†	1.16 ± 0.17	1.24 ± 0.16	3.41 ± 2.27
Tumor/muscle†	9.09 ± 1.55	6.57 ± 1.72	6.05 ± 0.92[c]
Tumor/liver†	2.39 ± 0.30	3.19 ± 0.48[c]	7.13 ± 3.52

* Animals killed 4 hr after ^{67}Ga administration.
† Mean of ratios for individual mice.
‡ n = Number of animals.
§ Significance at t-test (all compared with controls): [a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$.

agents to human tumor has obvious clinical implications. The increase reported here in the uptake of ^{67}Ga in tumor relative to normal tissues, produced by an i.v. injection of propranolol HCl, is thought to be the first time that this has been achieved by a vasoactive agent. The increased tumor perfusion is thought to be due to the change in the relative perfusion of muscle and of other tissues due to the decreased cardiac output with peripheral vasoconstriction to maintain blood pressure. Tumor blood vessels lack smooth muscle and, therefore, lack the ability to respond to vasoactive agents.

In the mouse it was not possible to measure the actual cardiac output but the perfusion changes in all the normal tissues studied was similar to that expected from studies in larger animals and man. It would be expected that other physiologic mechanisms, such as increasing blood flow by gravity would have similar effects. It is rather surprising that propranolol HCl should produce an enhanced ratio of ^{67}Ga between the tumor and normal tissues since the effect on the blood flow is transient, whereas gallium is known to be protein bound in the circulation and, therefore, accumulates slowly, reaching maximum levels after about 8 hr. It would be expected therefore that prolonged administration of propranolol HCl after ^{67}Ga injection would have a more marked effect on the relative tumor concentration of ^{67}Ga. When propranolol HCl was injected i.v. at 10 mg/kg 3 hr before the injection of ^{67}Ga, it had no effect on the distribution of the radionuclide.

In further experiments using other vasoactive agents we had less success in increasing relative tumor perfusion. In fact, hydralazine hydrochloride administered in a concentration of 5 mg/kg 40 min before the perfusion measurement caused a significant decrease in tumor perfusion ($p < 0.001$) (unpublished data). This drug, which is a direct vasodilator, may have some use in those studies attempting to reduce tumor flow to starve a tumor of its nutrients and in hyperthermia to facilitate local heating of the tumor by reducing the rate of loss of local heat.

The increase in relative tumor perfusion produced by pentobarbitone sodium demonstrated by Zanelli et al. (*2*) in mouse sarcomas and carcinomas was not demonstrated by us in this tumor model (unpublished data). It is believed that this may be due to differences in tumor types and differences between mouse strains. It was also important to standardize experimental conditions since the reaction to handling and other stimuli can markedly alter tumor perfusion in particular when anesthetics are not employed.

CONCLUSION

This communication has shown that at least one vasoactive agent can enhance the ratio of the uptakes of a tumor localizing radiopharmaceutical between a mouse tumor and normal tissues. It is hoped that human tumors will behave in a similar way, enabling earlier and more accurate diagnosis of human cancer by radioactive imaging techniques.

REFERENCES

1. Hammersley PAG: Relative tumour enhancement of ^{67}Ga uptake by desferrioxamine. *Eur J Nucl Med* 9:467-471, 1984
2. Zanelli GD, Lucas PB, Fowler JF: The effect of anaesthetics on blood perfusion in transplanted tumours. *Br J Cancer* 32:380-390,1975
3. Hammersley PAG, Taylor DM: The effect of the administration of iron on gallium-67 citrate uptake in tumours. *Br J Radiol* 53:563-571, 1980
4. Sapirstein LA: Regional blood flow by fractional distribution of indicators. *Am J Physiol* 93 (1):161-168, 1958