

Protamine as an Anti-Angiogenesis Agent for Cancer Therapy: Blood Coagulation of Tumor Feeding Vessels Measured by MRI

Min-Ying Su, Michael K. Samoszuk², Yi Song, and Orhan Nalcioglu

Health Sciences Research Imaging Center and ²Department of Pathology, University of California, Irvine, CA 92697

Purpose

Development of anti-angiogenic therapeutic agents has become increasingly important for cancer treatment since the role of angiogenesis in cancer development has been emphasized. One important aspect of the potential agents is the selectivity of action on the neovasculature, but not on the mature vessels. Mast cells are involved in the development of type I hypersensitivity reactions and are widely distributed in tissues. Many types of human and animal carcinomas, however, have been shown to accumulate large numbers of degranulating mast cells at the edges of the tumor and in the fibrovascular stroma within the tumor mass. Mast cell infiltration is significantly greater within human breast cancers than in benign breast lesions (1,2). Protamines are low-molecular weight proteins that are rich in arginine and strongly cationic. In the presence of heparin, which is strongly acidic, protamines and heparin rapidly form a stable salt in which the anticoagulant activity of heparin is neutralized (3). Because of the extensive degranulation of mast cells with deposition of heparin in adenocarcinomas, we hypothesize that protamine would be localized near the mast cells at the edges of the tumor, leading to coagulation of the tumor feeding vessels in mammary adenocarcinomas. Therefore, it may be a potential anti-angiogenic therapeutic agent for cancer treatment. To test the hypothesis, we compared the kinetics of Gd-DTPA before and after administration of protamine.

Methods

The imaging study was performed on a GE 1.5T Signa scanner. Five rats were studied. R3230 AC adenocarcinoma was inoculated subcutaneously into the thigh of the rats. The study was conducted at 4 weeks after the implantation, when the size of the tumor reached 2.0 cm in diameter. The animals were anesthetized with Ketamine (50mg/kg) and Rompun (5 mg/kg), then a 25 gauge butterfly needle was inserted into the tail vein for injection of agents. T2-weighted images were first acquired as localizer, then 6 slices from liver, kidney, tumor, and muscle were selected for dynamic studies. Dynamic T1-weighted images were taken with a spin echo pulse sequence with TR/TE= 200/12 ms. Gd-DTPA (0.1 mmol/kg) was first injected, and the kinetics acquired for 13 min. Thirty minutes after injection of Gd-DTPA, 3 mg protamine was injected. Then two more doses of 3 mg protamine were injected into the rat at 20 minutes intervals. Twenty minutes after injection of the third dose, another dose of Gd-DTPA (0.1 mmol/kg) was injected, and its kinetics was again measured for 13 minutes. After the study was finished, the animals were sacrificed for assessment of degree of bleeding in the tumor.

Results

Four out of the 5 rats survived in the study, indicating that although the protamine was given in 3 fractions, the total dose might be too toxic. The tumors of the remaining 4 rats all showed a dramatic response to the treatment. Figure 1 shows an example of the kinetics taken before and after protamine treatment from the liver, tumor, and muscle of one rat. Although both doses of Gd-DTPA were injected into the rat as a very fast bolus, in the kinetics taken from all three tissues, one can note a delay in the rising phase and a decreased peak enhancement after the treatment. However, in the liver and muscle the enhancement can reach to the pre-treatment value as time passes, indicating that although there is a delay eventually the contrast agent can be delivered to the entire extracellular space. In the tumor the delay was more pronounced, and the magnitude of enhancement was much lower than the pre-

treatment level. The percentage change of the enhancement in the pre and post treatment kinetics at 3 and 13 minutes after injection was calculated and summarized in table 1. After the animal was sacrificed, the tumor was assessed for the degree of bleeding. No blood was draining out as the tumor was cut open.

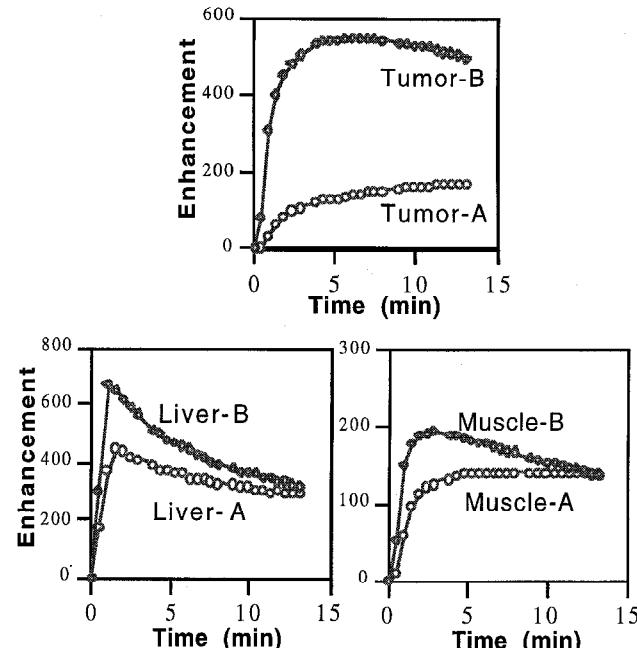


Figure 1. The enhancement kinetics of Gd-DTPA in tumor, liver, and muscle of one rat acquired before (B) and after (A) administration of protamine. The change was more pronounced in tumor than in the other normal tissues.

	Tumor	Liver	Muscle
3 min	- (77±16)% *	- (27±10)% *	- (32±12)% *
13 min	- (64±21)% *	- (7±11)%	+ (4±18)%

Table 1. The percentage change of the post-treatment enhancement compared to the pre-treatment value at 3 and 13 minutes after injection of Gd-DTPA. * means significant.

Discussion

In the bio-distribution study of biotinylated protamine, we observed that protamine was localized at the periphery of the tumors, possibly due to the presence of mast cells in this adenocarcinoma model. Therefore, when the protamine was injected intravenously it could localize at the edge of the tumor, bind with the deposited heparin and causes coagulation of the blood. As these feeding vessels were damaged, the subsequently injected contrast agent could not be delivered into the tumor to show strong enhancement. The effect was more pronounced in the tumor than normal tissues, suggesting that protamine might be a potential anti-angiogenic therapeutic agent.

References

1. Aaltomaa S, Lippinen P, Papinaho S, Kosma VM. Mast cells in breast cancer. *Anticancer Research* 13: 785, 1993.
2. Kankkunen JP, Harvima IT, Naukkarinen A. Quantitative analysis of tryptase and chymase containing mast cells in benign and malignant breast lesions. *Int J Cancer* 72: 385-388, 1997.
3. Promamint sulfate. In *Physician's Desk Reference*, Medical Economics Publishing n 1526 1997 1