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Streptavidin and Biotin as Potential Tumor Imaging Agents

TO THE EDITOR: We have read with interest the recent article by Hnatowich et al. (*1*) who have reported imaging of conjugated beads with indium-111-labeled streptavidin and biotin. We also have recent experience using these two agents and the purpose of our experimental study was to use the strong affinity of streptavidin to biotin ($K_d = 10^{-15}M$) to improve tumor imaging.

TABLE 1
Tumor/Organ Ratios in Nude Rats with Solid HeLa Cell Tumors 48 hr After Injection of [^{125}I]Streptavidin

Group*	1	2	3
Tumor/blood	2:2	1:0	1:9
Tumor/kidney	0:1	0:2	0:1
Tumor/liver	0:7	0:3	0:6
Tumor/lung	2:6	1:8	1:8
Tumor/heart	4:1	2:8	2:9
Tumor/intestine	4:4	3:1	3:1
Tumor/muscle	9:1	5:4	5:6

* Two rats per group.
Group 1: Anticytokeratin-biotin, [^{125}I]streptavidin.
Group 2: Nonspecific IgG-biotin, [^{125}I]streptavidin.
Group 3: [^{125}I]streptavidin.

Biotin was conjugated to anticytokeratin antibodies and injected i.v. into rats with solid HeLa cell tumors containing cytokeratin as a tumor-associated antigen. Three days later, ^{125}I-labeled streptavidin was injected i.v. The uptake of radioactivity was detected by scintigraphy as well as by radioactivity determination in tumor and various organ tissues by means of a well counter. Tumor/tissue ratios from the bled and

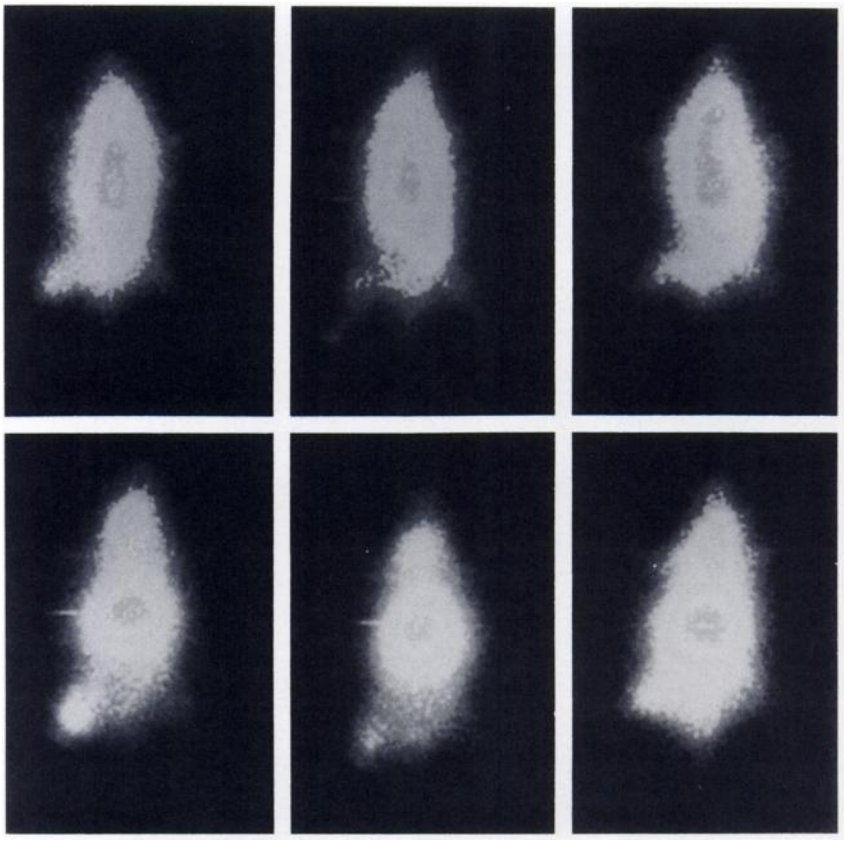

FIGURE 1
Whole-body images of nude rats bearing HeLa cell tumors on their left hind leg and injected i.v. with (125I) streptavidin. Images obtained 1 hr and 48 hr after injection of the radiolabeled streptavidin. A: Anti-cytokeratin-biotin, (125I) streptavidin—1 hr postadm. B: Nonspecific IgG-biotin, (125I) streptavidin—1 hr. C: (125I) streptavidin,—1 hr, D: see A—48 hr, E: see B—48 hr, F: see C—48 hr.

saline-perfused animals were calculated in order to establish the tumor-specific streptavidin binding. Control experiments were performed with biotin-conjugated nonspecific IgG or [^{125}I]streptavidin.

The results demonstrated that the tumor could be localized within 60 min after injection of radiolabeled streptavidin, whereas imaging was not possible before 3 days using the radiolabeled complete antibody (*2*). In further controls we demonstrated that there was some additional binding of streptavidin to the organs of the animals as well as to the tumor (Fig. 1, Table 1).

Using the biotin-streptavidin system, the tumor/blood ratio was higher compared to that after application of specific complete antibody (*3*).

In conclusion, immunoscintigraphy using radiolabeled streptavidin is a very rapid procedure for localizing tumors targeted with biotin-conjugated antibodies. The bond formation is specific and stable in vivo. Our results suggest that imaging and therapy studies may be substantially improved through the use of streptavidin labeled with high energy and short half-life isotopes.

A serious limitation to the system might be the high uptake of radioactivity in kidney and liver which is probably caused by biotin pools in these organs. Furthermore, the biologic half life of the administered antibody must be considered, since a portion of the injected [^{125}I]streptavidin will bind to biotinylated antibodies in the circulation of the animal before having reached the tumor. Application time as well as the administered quantity of [^{125}I]streptavidin have to be optimized. The use of F(ab)$_2$ fragments might overcome this problem since they clear from the circulation more rapidly.

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Leukocyte Labeling with Technetium-99m Tin Colloids

TO THE EDITOR: We draw attention to a recent publication by Mock and English in the *Journal* (*1*) discussing leukocyte labeling with technetium-99m (^{99m}Tc) tin colloids.

The authors state that they use a stock solution of stannous fluoride 0.125 mg/ml and sodium fluoride (1.00 mg/ml) (*1*), in an attempt to reproduce our method (*2*). We have never published a method involving a stock solution combining both starting materials but instead we use two individual solutions of stannous fluoride and sodium fluoride which are mixed together immediately before addition of sodium pertechnetate (*2,5*). This produces leukocyte labelling efficiencies in excess of 80% with strong leukocyte binding. The use of lyophilized tin colloid to label leukocytes (*4*) was evaluated by Danpure (*3*) and the lyophilized combined stannous fluoride and sodium fluoride radiopharmaceutical gave inconsistent and unsatisfactory results. Mock and English (*1*) used a frozen form, although chemically identical, and the poor leukocyte labeling efficiency is further evidence that the tin colloid is unsuitable if sodium fluoride and stannous fluoride are combined together in kit form or stock solution.

Additionally, the colloid preparation of Mock and English involved mixing 1 ml stannous stock solution with 1 ml sodium pertechnetate instead of 1 ml stannous and sodium fluoride solution and 5 ml sodium pertechnetate (*2,4,5*). We obtained all our results with the latter molar concentration.

We would refer the authors to our recent publication that discusses the factors affecting ^{99m}Tc leukocyte labeling by phagocytosis (*5*), which emphasizes the need for precise control of the variables in colloid preparation, particularly colloidal particle size, to produce satisfactory leukocyte labeling efficiency.

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REPLY: We appreciate the opportunity to respond to the above letter from Hanna, Lomas and Sullivan. Although Hanna and colleagues state that they "have never published a method involving a stock solution combining both starting materials," we quote from their own cited method (*1*), "After