FORMALDEHYDE-TREATED ^{131}I-ALBUMIN: A POSSIBLE BRAIN-TUMOR SCANNING AGENT

John L. Mego and J. Donald McQueen

Baltimore City Hospitals and The Johns Hopkins University School of Medicine, Baltimore, Maryland

Of the many scanning agents being used for brain-tumor localization, ^{131}I-albumin offers the highest sustained levels of radioactivity in experimental tumors (*1*). However, this material has the major disadvantages of having a relatively long biological half-life and delivering high levels of radioactivity to the blood stream. ^{131}I-albumin has been treated with heat to form micro- or macroaggregates which are useful in lung, liver and spleen scanning (*2–6*), and at least one report has appeared on the possible use of these preparations in brain scanning (*7*).

We have found that treatment with buffered (*p*H 9) formaldehyde does not alter the solubility or general appearance of human radioiodinated serum albumin, but the material is rapidly cleared from the blood stream and taken up by the liver when injected into mice (*8*). The formaldehyde-treated protein is quickly degraded within subcellular particles in the liver, and the radioactive fragments are excreted. In the present study, we have tested human serum ^{131}I-albumin treated at various *p*H in tumor-bearing mice for possible usefulness as brain-tumor scanning agents.

MATERIALS AND METHODS

We obtained the human ^{131}I-albumin from Abbott Laboratories and treated it with formaldehyde by adding one part protein solution (10 mg/ml) to a mixture of one part 37% formaldehyde (Baker Analyzed Reagent) and one part 0.5 M phosphate (*p*H 7, 7.5 or 8) or carbonate (*p*H 9 or 10) buffer. The mixture was allowed to react in the refrigerator for 3 days before being dialyzed for 24 hr against 0.9% NaCl. The proteins can also be prepared by iodination after formaldehyde treatment. Treatment of the labeled albumin with formaldehyde at *p*H 7–10 did not cause detectable aggregation or precipitation of protein. The materials could be passed through a Millipore filter with 0.45-micron pores, and they were not sedimented by centrifugation at 39,000 G for 1 hr. No precipitation or aggregation took place during refrigeration for as long as a week after preparation. Indeed, it has been reported that proteins become more resistant to heat, enzymes and chemicals after treatment with formaldehyde (*9*).

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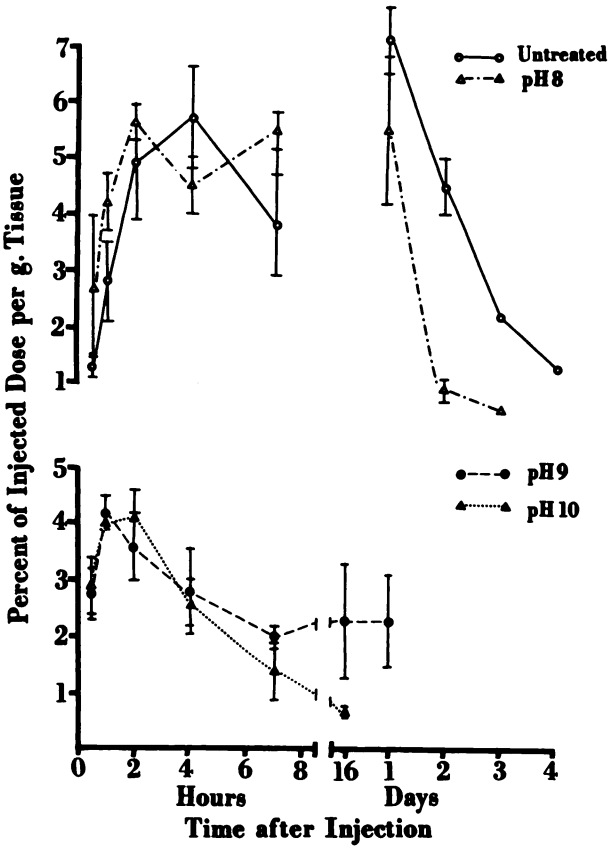

FIG. 1. Uptake of ^{131}I-albumin treated with formaldehyde at pH 8, 9, or 10 in tumor compared with untreated protein in turn. The vertical lines indicate twice the standard error.

We injected male C-57 mice bearing the transplantable Zimmerman ependymoblastoma with 0.1 ml (about 3 μc, 0.3 mg) treated or 0.1 ml untreated (diluted 1:3 with saline) labeled protein via the tail vein using a 30-gage needle and a 0.25-ml syringe. The mice were anesthetized with ether, and a sample of blood was removed from the heart. The animals were then perfused with saline through the heart until the liver was blanched. Tissues were removed, weighed and counted in a well counter for 10 min. The counts were calculated for 1 gm of tissue (or 1 ml blood) and normalized to a 25-gm mouse weight.

RESULTS

Variations in uptake of the various protein preparations were more pronounced in tumor than in the other tissues. Figure 1 shows the uptake of ^{131}I-albumin treated at *p*H 8, 9 or 10 in tumor compared with untreated protein. Protein treated at *p*H 8 was taken up to about the same extent as untreated albumin except that the levels declined somewhat more rapidly after 1 day. The radioactivity increased up to about 2 hr and then leveled off. At *p*H 9 or 10, however, the labeled albumin began to leave the tumor after 2 hr and did not reach the concentrations attained with untreated material.

The uptake of the various proteins into liver and kidney are shown in Figs. 2 and 3. Although high *p*H caused elevated levels in these tissues initially, the radioactivity was quickly cleared, and within 2 hr the concentrations were down to those obtained with untreated protein. After 2–3 days the levels were about 1 – 2% of the injected dose per gram.

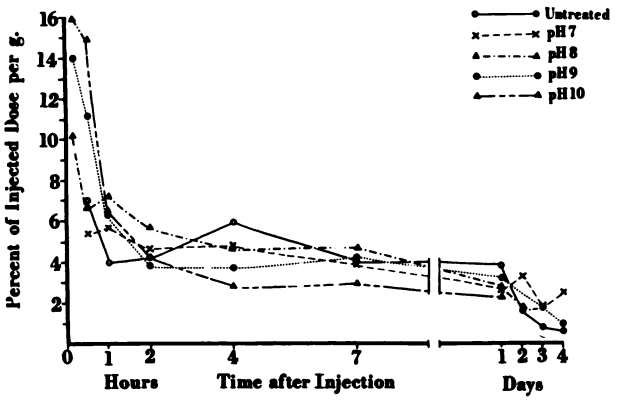

FIG. 2. Levels of radioactivity in liver resulting from injection into mice of ^{131}I-albumin treated at pH 7, 8, 9, or 10 compared with untreated protein.

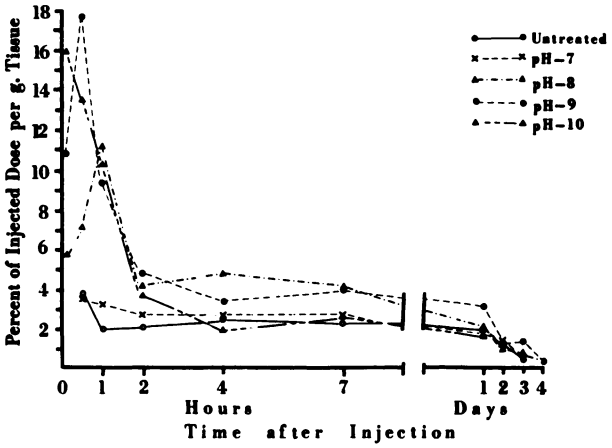

FIG. 3. Levels of radioactivity in kidney from ^{131}I-albumin treated at pH 7, 8, 9, or 10 compared with untreated protein.

Figure 4 shows the clearance from the blood stream of the various proteins. As early as 30 min after injection, levels of albumin treated at *p*H 8 were significantly lower than the untreated protein. After 2 hr the levels were at least half those obtained with the control, and this ratio was main-

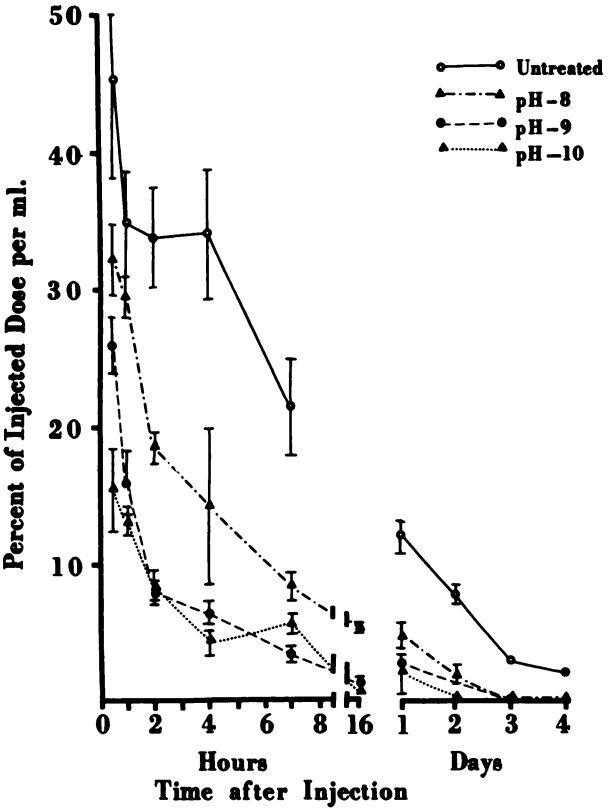

FIG. 4. Clearance of formaldehyde-treated ^{131}I-albumin (pH 8, 9, or 10) from the blood stream of mice compared with untreated protein. Vertical lines indicate twice the standard error.

tained up to 2 days after injection. After 2 days the radioactivity declined to negligible levels although a significant quantity of the untreated albumin persisted up to 4 days. Higher *p*H resulted in low blood radioactivity concentrations, but there was no apparent difference in the blood levels of albumin treated at *p*H 9 or 10.

The labeled albumin treated at *p*H 7 and shown in Figs. 2 and 3 (or at *p*H 7.5 or with *p*H 10 buffer alone, not shown in these figures) behaved similarly to untreated protein with respect to clearance from the blood stream and uptake into tissues.

We also determined the uptake of formaldehyde-treated ^{131}I-albumin in muscle and brain. In general, the muscle uptake paralleled the tumor levels, but the concentrations remained between 1 and 2% of the injected dose per gram tissue with all the preparations. Clearance from muscle was more rapid with the material treated at *p*H 9 or 10: in 4 hr the levels began to drop and were negligible by 16 hr. Slightly less uptake of the formaldehyde-treated albumin occurred in the brain, resulting in somewhat elevated tumor-to-brain ratios with *p*H-8-treated materials. Tumor-to-brain ratios with *p*H-9 or *p*H-10-treated albumin were about the same as those obtained with untreated protein because of decreased uptake in tumor. Tables 1 and 2 show concentrations of radioactivity in muscle and brain and tumor-to-brain and tumor-to-muscle ratios for untreated ^{131}I-albumin and *p*H-8-treated ^{131}I-albumin.

DISCUSSION

Formaldehyde reacts with amino groups of proteins to form methylene bridges across peptide chains (*10*). This reaction can be favored by alkaline *p*H due to the high *p*K (about 10) of the ϵ-amino group of lysine. At *p*H 8 most of the amino groups would be in a protonated (charged) state, and only a few would be reactive. At *p*H 9 or 10, more groups would occur in a reactive uncharged state. Higher *p*H would also expose more amino groups to the action of formaldehyde by causing the proteins to unfold partially. Some changes in secondary or tertiary structure probably occur which cause the cells of the liver and kidney to recognize the formaldehyde-treated serum albumin as abnormal.

Reaction with free amino groups decreases the number of positive charges, tending to produce a more acidic protein with a lower isoelectric point (*9*). This alteration in charge may be partly responsible for the uptake in the liver and kidneys although it may be possible that methylene bridges are formed between protein molecules resulting in aggregates of two or more molecules. Fraenkel-Conrat and Olcott (*11*) have shown that treatment of the protamine, salmine, with formaldehyde results in a large increase in molecular weight, suggesting that intermolecular bonds are formed. X-ray studies by Low (*12*) indicate that human plasma albumin has the shape of a prism 145 Å long and 22 × 50 Å thick. Since the formaldehyde-treated proteins are easily passed through Millipore filters with 4,500-Å pores, extensive aggregation (more than 100–200 molecules) does not appear likely. Reaction with formaldehyde does not affect the hydrolysis of the protein in the liver because it is quickly removed from the organ (Fig. 2, ref. *8*).

In a previous publication, we noted that the uptake of differentially coupled ^{74}As-arsono-poly-L-lysine into tumor was related to the clearance rates from the blood stream (*13*). Specifically, enhanced

TABLE 1. CONCENTRATIONS OF RADIOACTIVITY WITH UNTREATED ^{131}I-ALBUMIN

Time	No. of mice	% injected dose/gm		Tumor/brain	Tumor/muscle
		Muscle	Brain		
½ hr	5	1.1 (0.2)	0.21 (0.07)	6.1	1.2
1 hr	4	1.3 (0.2)	0.41 (0.06)	6.8	2.2
2 hr	5	0.9 (0.1)	0.69 (0.15)	7.1	5.4
4 hr	4	1.9 (0.4)	0.34 (0.04)	16.8	3.0
7 hr	5	1.6 (0.6)	0.22 (0.003)	17.3	2.4
24 hr	5	1.2 (0.1)	0.27 (0.04)	25.6	5.8

Standard errors are indicated in parentheses.

TABLE 2. CONCENTRATIONS OF RADIOACTIVITY WITH pH-8-TREATED ^{131}I-ALBUMIN

Time	No. of mice	% injected dose/gm			
		Muscle	Brain	Tumor/brain	Tumor/muscle
½ hr	4	1.3 (0.4)	0.30 (0.04)	9.0	2.1
1 hr	7	1.6 (0.7)	0.34 (0.07)	12.4	2.6
2 hr	4	1.4 (0.03)	0.35 (0.04)	15.4	3.9
4 hr	5	1.2 (0.3)	0.21 (0.02)	21.4	3.8
7 hr	4	1.5 (0.1)	0.18 (0.05)	30.6	3.7
24 hr	5	1.2 (0.3)	0.20 (0.06)	27.5	4.7

Standard errors are indicated in parentheses.

uptake resulted if blood concentrations were maintained. The results of our present study also suggest this relationship because protein treated at high *p*H was rapidly cleared from the blood stream and uptake into tumor was correspondingly depressed.

However, the results obtained with ^{131}I-albumin treated at *p*H 8 appear to be an exception to this observation. With this material, levels in the tumor during the first 24 hr were the same as those obtained with untreated protein although the blood levels were at least 50% less. The relatively high uptake in tumor of this protein despite the more rapid clearance from the blood may be caused by the blood levels which were high enough initially to permit saturation of the tumor. Therefore more elevated blood levels, such as those obtained with untreated protein, would not increase the tumor concentration appreciably. The tumor may be capable of greater absolute uptake if the injected protein dose is increased, but the blood levels would also be elevated correspondingly, and the percentage figures would be unchanged.

The chemical treatment of ^{131}I-albumin appears to have several advantages over physical agents such as heat. The major advantages are a more precise degree of control, better reproducibility and the possibility of obtaining graded effects that cannot be reached with physical agents. Treatment with formaldehyde results in a labeled protein which is more stable to denaturation and is completely soluble and free of precipitates. Therefore it is not necessary to filter or centrifuge the material. The treatment procedure is simple, and the properties of the product at a given *p*H are constant from preparation to preparation. Other chemical reactions with albumin are possible (acetylation, deamination) which may prove superior to the formaldehyde reaction.

There are several ways in which the time needed for preparing the proteins used in this study can be shortened. As we noted earlier, the albumin can be iodinated after treatment with formaldehyde; we have recently accomplished this procedure successfully. The time needed to complete the formaldehyde treatment has not been determined, but the reaction should proceed more rapidly at temperatures higher than 5°. By using gel filtration to remove formaldehyde and buffer, one can eliminate the time-consuming dialysis period.

SUMMARY

We have evaluated formaldehyde-treated ^{131}I-albumin as a possible brain-tumor scanning agent in ependymoma-bearing C-57 mice. The rate of clearance from the blood stream and uptake intc tissues differed according to the *p*H at which the protein was treated with formaldehyde. Treatment at *p*H 7 or 7.5 did not appreciably change the uptake patterns. A *p*H 8 resulted in blood radioactivity levels about half those obtained with untreated protein, but the tumor levels were approximately the same. Higher *p*H of 9 and 10 resulted in rapid clearance from the blood stream and uptake into liver; in this case, tumor levels were reduced to about two thirds of the control. The results suggest that ^{131}I-albumin treated at *p*H 8 with formaldehyde may be superior to untreated or heat-aggregated albumin as a tumor scanning agent.

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