
Effect of Bicarbonate on Stability of the Gallium-Transferrin Complex

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When gallium citrate is injected intravenously the gallium is rapidly transchelated to transferrin. If the stability of the gallium-transferrin association is sufficiently strong it should be a good macromolecular tracer suitable for quantitative measurements of vascular permeability. Studies of the stability of the gallium transferrin complex in human plasma and serum were done using ultrafiltration and dialysis. It was found that the stability was sensitive to bicarbonate level. Above 13 mM of bicarbonate, 97%–99% of the gallium is bound to molecules >10,000 MW by ultrafiltration. However, at 10 mM binding is 95% and at 7 mM 83%. Similar results were obtained with dialysis. This suggests the gallium-transferrin complex may not be sufficiently stable for quantitative measures of vascular permeability, particularly if the bicarbonate concentration is low.

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A fundamental assumption of all tracer kinetic studies of vascular permeability is that the label remains bound to the tracer molecule. When gallium-citrate is injected intravenously it is assumed that the ionic gallium, which is weakly chelated to citrate, promptly transfers to transferrin, where it remains firmly bound. Recent work by Brunetti et al. (1) question the validity of this assumption and suggest that the relative instability of the gallium-transferrin (Ga-TF) complex may preclude its use for measurement of vascular permeability using positron emission tomography (PET). However, other investigators (2) feel that Ga-TF is appropriate for use with PET and have used it with apparent success. The stability of the Ga-TF complex is essential for accurate and reproducible measures of vascular permeability since unbound gallium is likely to diffuse rapidly across endothelium, resulting in inaccurately high estimates of vascular permeability.

The binding of gallium to transferrin is known to depend upon pH and on the concentration of bicarbonate. Tsan and coworkers (3) found the presence of the bicarbonate

ion improved the binding of gallium to transferrin. However, Tsan used low concentrations of bicarbonate [HCO_3^-] and high concentrations of transferrin, compared to physiologically normal levels. The purpose of this study was to examine the binding of gallium to transferrin within the physiologic range of bicarbonate concentrations in human serum (normal is 24 mM; range is 8 to 30 mM) and to determine the effect bicarbonate has on the stability of the binding.

MATERIALS AND METHODS

Carrier-free ^{67}Ga -citrate was purchased from Medi-Physics, Chicago, IL. Iodine-131 (ICN Radiochemicals, Irvine, CA) was labeled to human serum albumin by a colleague in the Division of Nuclear Medicine using a standard, non-sterile chloramine-T iodination technique (4). Free iodine was removed prior to each use by passing the protein solution through Sephadex G-25M pre-packed in PD-10 disposable columns (Pharmacia Fine Chemicals, Piscataway, NJ). All samples were counted in a gamma counter (Packard Instrument Co., Chicago, IL). Human plasma or serum was obtained from normal volunteers 1 hr prior to experimentation. Whole blood was centrifuged at $1,000 \times G$ for 10 min to separate serum or plasma from red blood cells. Plasma was obtained using a heparinized syringe.

Ultrafiltration was performed using Millipore Ultrafree-MC filter units (Millipore, Bedford, MA) with 10,000 molecular weight low-binding PLGC membranes. These filter units consist of 45-mm microfuge tubes containing a removable, nonadhesive, polypropylene filter with a maximum volume of 0.4 ml. Gallium-67-citrate or ^{131}I -albumin was incubated in 1 ml of normal human serum or plasma at room temperature for 10 min. An aliquot (0.2 ml) was then transferred to the filter unit and centrifuged at $8,000 \times G$ for 20 min. After centrifugation, the filter and cone of the unit were separated and counted individually.

Dialysis was performed using Spectrapor seamless semi-microtubing with 12,000–14,000 molecular weight cutoff (Spectrum Med. Inc., Los Angeles, CA). An aliquot (0.5 ml) of serum containing ^{67}Ga and ^{131}I -albumin was dialyzed against 14 ml of normal serum at 37°C. Samples were taken at 0.5, 1, 1.5, and 3 hr after initiation of dialysis. All dialysis studies were done with serum.

RESULTS

Ultrafiltration of ^{67}Ga in plasma or serum showed 97%–99% of the radioactivity retained by the filter at bicarbonate concentrations above 13 mM. However, below 13 mM

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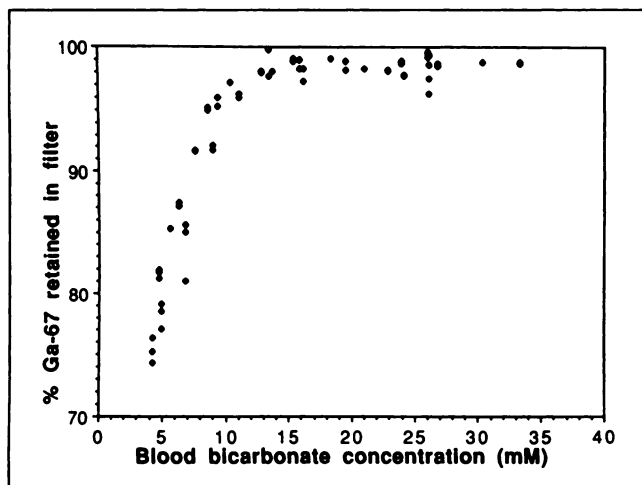


FIGURE 1. Ultrafiltration of ^{67}Ga in human plasma. Ultrafiltration of 0.2 ml of human serum or plasma containing ^{67}Ga was performed at $8,000 \times G$ for 20 min using Millipore Ultrafree-MC filter units with 10,000 molecular weight cutoff. At bicarbonate concentrations above 13 mM, 97%–99% of ^{67}Ga remains in the filter, presumably bound to transferrin. At bicarbonate concentrations less than 13 mM, the percent gallium retained in the filter decreases steadily, suggesting a higher percent of free gallium or gallium bound to lower molecular weight molecules.

$[\text{HCO}_3^-]$ the fraction of ^{67}Ga remaining in the filter dropped to 95% at 10 mM and 83% at 7 mM (Fig. 1). Ultrafiltration rates were similar for plasma and serum.

Passage of ^{67}Ga through the dialysis membrane occurred significantly more rapidly at low bicarbonate concentrations (2–7 mM) than it did at physiologically normal levels (24–30 mM). After 1.5 hr, 65% of the ^{67}Ga had passed through the membrane at low bicarbonate levels, while only 25% passed through at normal levels (Fig. 2). The clearance of ^{131}I -albumin was not affected by varying concentrations of bicarbonate with 99% of ^{131}I -albumin staying inside the dialysis membrane after 3 hr.

DISCUSSION

Measurement of the percent of gallium bound to large molecular weight molecules, presumably transferrin, as a function of bicarbonate concentration demonstrates the existence of a threshold concentration of 13 mM bicarbonate necessary for stable binding of gallium by transferrin. Dialysis shows that the ^{67}Ga label, which is expected to be bound to transferrin, an 80,000 molecular weight protein, diffuses across a semipermeable membrane more easily than ^{131}I -albumin, a 68,000 molecular weight protein. This is most likely because there is a significant fraction of either free gallium or gallium bound to small molecules that rapidly diffuses across the membrane. Otsuki et al. (5) in studies with rats also found Ga-TF, as well as Fe-TF, cleared more rapidly from the blood than albumin. These investigators examined the uptake of labeled transferrin into various tissues using three different tracers, ^{59}Fe , ^{111}In and ^{68}Ga . Although each metal is chelated by trans-

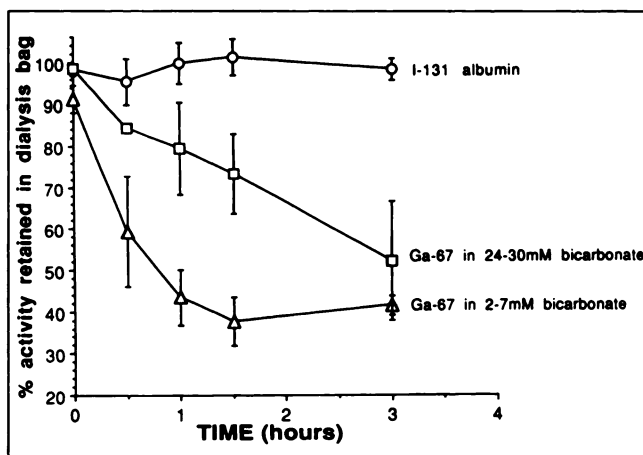


FIGURE 2. Dialysis of ^{67}Ga in human serum. Dialysis of 0.5 ml human serum containing ^{67}Ga and ^{131}I -albumin in Spectrapor 12,000–14,000 molecular weight cutoff microtubing against 14 ml normal serum at varying bicarbonate concentrations shows a higher leakage of gallium across the membrane at lower bicarbonate concentrations. Bicarbonate concentrations given are the range of concentrations measured throughout the 3-hr experiments. In general, the $[\text{HCO}_3^-]$ rose throughout the experiment. Gallium-67 error bars represent the standard error of three experiments. Iodine-131-albumin was dialyzed along with ^{67}Ga and error bars represent the standard error from both low and high bicarbonate concentrations.

ferrin, the subsequent kinetics were found to be somewhat different. Indium-transferrin seems to be the most stable metal-transferrin complex with both Fe-TF and Ga-TF showing more rapid plasma clearance and higher uptake into tissues. This observation is consistent with lower stability of iron and gallium transferrin complexes.

The threshold bicarbonate concentration of 13 mM necessary for the binding of gallium by transferrin is significant since, although most patients have a bicarbonate concentration around 24 mM, the $[\text{HCO}_3^-]$ of sick patients can drop well below 13 mM. Unbound gallium will quickly diffuse across the endothelium of these patients resulting in an apparent increase in vascular permeability. In addition, there may be areas where the local $[\text{HCO}_3^-]$ level is low, resulting in local relative accumulation of gallium. This may explain the high uptake of gallium into resting muscle seen by Otsuki et al. (5).

Mintun and co-workers (2) found that the calculated values of the pulmonary transcapillary escape rate (PTCER) were higher using ^{68}Ga than labeled albumin. They did not, however, consider this difference to be of significance, although the higher PTCER for Ga-TF is probably a result of the rapid movement of the small molecular weight fraction across vascular endothelium.

The results of the present study suggest that variation may occur between patients and even between two scans of the same patient performed on different days, because of possible changes in bicarbonate levels. While reliable measurements can be made under normal conditions, the

possibility of such variance is a potential problem with this technique.

Another use of gallium in clinical nuclear medicine is for diagnosis and localization of tumors. Uptake of gallium by tumors has been thought to be secondary to the increased number of transferrin receptors that bind and internalize the Ga-TF complex (6). Gallium is then transferred to other subcellular macromolecules such as ferritin and stays firmly bound inside the cell. Because malignant tumors are often hypoxic, it is possible that the localization of gallium could also be facilitated by the release of gallium from transferrin under the acidic conditions of a tumor followed by binding to ferritin proteins within the cell. This hypothesis is supported by the observation of Otsuki et al. (5) that the Ga-TF complex concentrates to some extent in Walker 256 tumors in the rat, while In-TF does not. In any case, Ga-TF is certainly an inappropriate tracer for measuring vascular permeability in tumors because of the relatively high level of free gallium (or a low molecular weight complex), because of the possibility that regional hypoxia may lead to low bicarbonate levels and excessive release of gallium from the complex, and finally because the binding of Ga-TF to the increased number of transferrin receptors in tumor would be indistinguishable from increased vascular permeability.

CONCLUSIONS

The present work, showing relatively rapid passage of ^{67}Ga nominally bound to transferrin through ultrafiltra-

tion and dialysis membranes, supports the concept that Ga-TF is an unstable complex and is not well suited for quantitative studies of vascular permeability. This instability arises, at least in part, from a sensitivity of the complex to bicarbonate concentrations. Under low bicarbonate concentrations gallium disassociates from transferrin and can then pass more easily across the endothelium. This phenomenon may also be involved in the localization of gallium in hypoxic tumors.

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