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EDITORIAL

Technetium-99m-HMPAO Retention and the Role of Glutathione: The Debate Continues

Many patients presenting with primary brain tumors face a grim prognosis due to the inability to control local disease (*1*). Secondary brain tumors present similar therapeutic difficulties, although survival probably depends more on the extent of extra-cerebral disease (*2*). However, as more aggressive antineoplastic regimens begin to demonstrate greater control of systemic disease, survival may depend on adequate treatment of central nervous system secondaries.

Tumor perfusion may play an important role in the success of antineoplastic therapies such as radiotherapy and chemotherapy due to their dependence on adequate blood flow for oxygenation and drug transport, respectively. A noninvasive means of studying brain tumor blood flow should help us to improve our understanding of the role perfusion plays in brain tumor therapy and prognosis.

rCBF TRACERS

Regional cerebral blood flow (rCBF) tracers for use with single-photon emission tomography (SPECT) must possess, amongst other characteristics, a mechanism by which the activity extracted into the brain is fixed for a sufficient time to allow for image acquisition. In order to accomplish this, a "trapping" mechanism must exist. Ideally, a prerequisite for the trapping mechanism is that it is unaffected by pathology. It is then possible to begin to interpret tracer distribution images as true rCBF images in a range of disorders.

The ability to image regional rCBF using nondynamic SPECT was brought about by the introduction of the ^{123}I-labeled-amines; IMP (p-iodo-N-isopropyl amphetamine) (*3*) and HIPDM (N,N,N,-trimethyl-N-[2-hydroxy-3-methyl-5-iodobenzyl]-1,3-propanediamine) (*4*). Both compounds demonstrated high first-pass extraction in the brain and prolonged retention. While the exact "trapping" mechanism of the amines remains uncertain (*5*), they have proved useful in aiding the diagnosis of both cerebrovascular and neurologic disorders (*6–8*).

The application of these early SPECT tracers excluded the study of neurooncology due to the reported lack of a functional trapping mechanism in primary brain tumors (*9–11*), although there were reports of uptake in metastases to brain (*12,13*) and in isolated cases of primary brain tumors (*14*).

Technetium-99m-HMPAO

Technetium-99m-d,l-HMPAO (exametazime) is the first clinically available ^{99m}Tc-labeled rCBF tracer. This tracer exhibits high first-pass extraction in the brain and prolonged retention, making it suitable for SPECT imaging using non-dynamic systems (*15,16*). Initial studies in patients with brain tumors indicated that, unlike IMP and HIPDM, this new tracer was capable of localizing in primary brain tumors (*17*).

The cerebral retention of ^{99m}Tc-HMPAO is believed to involve the intracellular conversion of the hydrophobic Tc-HMPAO to a species which is incapable of rapid back diffusion. The proposed mechanism of this conversion is thought to involve interaction of ^{99m}Tc-HMPAO with glutathione (GSH) (18). This theory is based on the similarity in measured conversion rates of ^{99m}Tc-d,l-HMPAO (to a less hydrophobic species) when ex-

Received Apr. 4, 1991; revision accepted Apr. 23, 1991.
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posed to rat brain homogenates or aqueous solutions of GSH. The meso diastereomer of ^{99m}Tc-HMPAO demonstrated a slower conversion rate but similar rates for brain homogenate and aqueous GSH (*19*). Pharmacokinetic modeling of the human distribution and retention of the meso and d,l forms of ^{99m}Tc-HMPAO, using these experimentally derived conversion rates ("K3") for each, were shown to be in good agreement with measured human distributions (*19*). While these data support the hypothesis that GSH is responsible for intracellular trapping, such findings are not unequivocal proof.

GSH

GSH is a ubiquitous tripeptide, accounting for a third of the brain's total non-protein, acid-extractable nitrogen and at least 95% of the thiols in this fraction (*20*). GSH is involved in many cellular functions, it is a co-factor in the metabolism of xenobiotics, serves as a storage and intercellular transport form of cysteine, provides for the maintenance of protein sulfhydryl reduction and is involved in the protection of membrane lipids from peroxidation (*21*). In addition, GSH is reported to play a role in DNA synthesis, protein synthesis and amino acid transport as well as being a co-factor in several enzyme systems (*22*).

Since GSH is so intimately linked to a variety of metabolic functions and the detoxification of metabolic by-products, it seems likely that normal metabolically active tissue demanding high nutrient perfusion may also have high levels of GSH or GSH-related enzyme activity. Conversely, ischemic tissues which lack nutrient perfusion would be expected to exhibit low levels of GSH.

Data in the rat kidney indicates that induction of ischemia leads to rapid loss of GSH, secondary to a loss of ATP, thereby inhibiting GSH synthesis (*23*). In this case, a lack of synthesis coupled with rapid catabolism of renal GSH leads to GSH depletion. Similar studies indicate that reductions of GSH occur in rat brain as a result of ischemia (*24*). However, brain GSH turnover is slow compared to the kidney and other organs (*25*) and it is possible that transient drops in perfusion would not produce rapid changes in brain GSH levels.

It therefore seems likely that GSH levels may parallel blood flow in certain tissues and that GSH levels may be reduced in hypoxic tissues. Recent evidence for the existence of a carrier-mediated transport system for the blood-brain barrier transport of GSH (*26*) suggests that a component of intracellular GSH content may be related to interorgan transport (*25*), which could be flow-dependent. Hence, it may prove difficult to separate outflow from GSH content in normal brain tissue.

There are reports in the literature, however, which suggest that intracellular trapping may not wholely depend on the presence of intracellular GSH. El-Shirbiny et al. (*27*) reported a lack of correlation between organ GSH content and ^{99m}Tc-d,l-HMPAO uptake in the rat before and after glutathione depletion using diethyl maleate. Rowell et al. (*28*) studied the uptake of the ^{99m}Tc-meso-HMPAO in lung tumors, comparing uptake to lung tumor GSH levels. The driving hypothesis of the latter study was that the accumulation of ^{99m}Tc-meso-HMPAO was dependent more on GSH content than blood flow (*19*). No correlation could be demonstrated between ^{99m}Tc-meso-HMPAO uptake and tumor GSH concentration or tumor/normal lung GSH ratios in the small number of patients studied, although the intracellular GSH concentration in malignant lung tumors was generally higher than that of normal lung.

RELATIONSHIP BETWEEN GSH LEVELS AND d,1-HMPAO

In this issue of *The Journal of Nuclear Medicine*, Suess et al. describe the relationship between tumor GSH levels and ^{99m}Tc-d,l-HMPAO uptake (as measured by tumor/cerebellum ratios) in patients with primary and secondary brain tumors. They report a statistically significant correlation between tumor GSH levels and ^{99m}Tc-d,l-HMPAO uptake in both meningiomas and gliomas, giving further indirect support to the GSH "trapping" mechanism hypothesis. Conversely, no correlation could be demonstrated between GSH content in metastatic tumor deposits in the brain and ^{99m}Tc-d,l-HMPAO uptake. In addition, ^{99m}Tc-d,l-HMPAO uptake occurred in certain tumors even when tissue GSH levels were undetectable.

Two obvious findings are apparent in this report: ^{99m}Tc-d,l-HMPAO uptake into brain tumors often parallels GSH levels; and secondary tumors to the brain lack any such parallelism. Interpretation of these findings is not clear cut and may point to anatomic and physiologic differences between these two tumor groups based on their tissue of origin, the nature of their vascularization or blood supply and their biochemistry relative to GSH metabolism, including GSH synthesis and content and the activity of enzymes that utilize GSH as a substrate or co-factor.

The correlation of GSH with ^{99m}Tc-HMPAO uptake in primary brain tumors may reflect further subtleties of GSH metabolism that have yet to be considered. Recent data indicate that brain tumors possess elevated levels of an isoenzyme, of the glutathione-S-transferase (GST) family of enzymes, which catalyze the conjugation of reduced GSH to hydrophobic, electrophilic compounds (*29,30*). If GSH is indeed involved in the trapping of ^{99m}Tc-HMPAO, then it is not unlikely that the enzymes responsible for the conjugation of GSH to xenobiotics may play a role in this process. This may help explain the correlation found with primary brain tumors, but it does little to clarify the lack of relationship seen in metastases. Variable washout rates of the "converted" ^{99m}Tc-HMPAO species between secondary and primary brain tumors may also add to the discrepancy between GSH levels and observed uptake.

Further studies are required to gain a better understanding of the "trapping" mechanism of ^{99m}Tc-HMPAO in normal and neoplastic tissues. Determining the relationship between perfusion (using a freely diffusible tracer) and GSH tissue levels in normal and diseased tissues may be the first step in achieving this goal.

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