PET Imaging of Copper Trafficking in a Mouse Model of Alzheimer’s Disease

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ABSTRACT

Alzheimer's disease (AD) is a fatal neurodegenerative disorder characterised by progressive neuronal loss and cognitive decline. The lack of reliable and objective diagnostic markers for AD hampers early disease detection and treatment. Growing evidence supports the existence of a dysregulation in brain copper trafficking in AD. The aim of this study was to investigate brain copper trafficking in a transgenic mouse model of AD by PET imaging with copper-64 ($^{64}$Cu), to determine its potential as a diagnostic biomarker of the disorder.

Methods: Brain copper trafficking was evaluated in 6-8-month-old TASTPM transgenic mice and age-matched wild-type controls using the $^{64}$Cu bis(thiosemicarbazone) complex $^{64}$Cu-GTSM, which crosses the blood-brain barrier and releases $^{64}$Cu bioreductively into cells. Animals were intravenously injected with $^{64}$Cu-GTSM and imaged at 0-30 min and 24-25 h post-injection. The images were analysed by atlas-based quantification and texture analysis. Regional distribution of $^{64}$Cu in the brain 24 h post-injection was also evaluated via ex vivo autoradiography and compared to amyloid-β plaque deposition in TASTPM mice.

Results: PET image analysis demonstrated significantly increased (by a factor of approx. 1.3) brain concentration of $^{64}$Cu at 30 min (p<0.01) and 24 h (p<0.05) post-injection of the tracer and faster (by a factor of approx. 5) $^{64}$Cu clearance from brain (p<0.01) in TASTPM mice compared to controls. Atlas-based quantification and texture analysis revealed significant differences in regional brain uptake of $^{64}$Cu and PET image
heterogeneity, respectively, between the two groups of mice. *Ex vivo* autoradiography showed that regional brain distribution of $^{64}$Cu at 24 h post-injection did not correlate with amyloid-β plaque distribution in TASTPM mice.

**Conclusion:** The trafficking of $^{64}$Cu in brain after administration of $^{64}$Cu-GTSM is significantly altered by AD-like pathology in the TASTPM mouse model, suggesting that $^{64}$Cu-GTSM PET imaging warrants clinical evaluation as a diagnostic tool for AD and possibly other neurodegenerative disorders.

**Keywords:** Copper trafficking, Alzheimer’s disease, positron emission tomography, copper-64

**Abbreviations:** AD, Alzheimer’s disease; Aβ, amyloid-β; PET, positron emission tomography; APP, amyloid precursor protein; $^{64}$Cu-GTSM, glyoxalbis($\text{N}^4$-methyl-3-thiosemicarbazonato) copper (II); BBB, blood-brain barrier; ROI, region of interest; %ID/g, percentage of injected dose per gram.
INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia in the elderly. Its prevalence in an ageing global population is rapidly increasing, creating a major socio-economic burden. There is no satisfactory method for its early diagnosis and no effective treatment to halt or delay the cognitive decline experienced by patients. Currently, the most promising application of molecular imaging in AD is the visualisation of amyloid-β (Aβ) burden using positron emission tomography (PET) radioligands that bind specifically to Aβ plaques, such as the $^{11}$C-Pittsburgh compound B, $^{18}$F-Florbetaben, $^{18}$F-Florbetapir and $^{18}$F-Flutemetamol (1). Nevertheless, clinical data indicate that imageable Aβ plaque load alone is not an adequate predictor of disease progression (2,3); many subjects with high Aβ burden detected by PET did not subsequently develop AD, and there is a need for PET radiotracers for a wider range of molecular features of the disorder (4).

In recent years, there has been growing evidence associating changes in trafficking and distribution of trace metals, including copper, with the onset and progression of AD (5, 6). Whether this association is a cause or consequence of the pathology is unknown and the nature of the changes is poorly understood. Copper has been found to interact with key components of AD pathology (Aβ, amyloid precursor protein [APP], β-secretase 1, tau) and, as a result, is thought to contribute to amyloidosis, synaptic toxicity and oxidative stress (7-11). Aβ plaques have been described as “sinks” of iron, zinc and copper ions (12,13), which have been shown to enhance Aβ aggregation and
neurotoxicity (14, 15). It has been suggested that the association of copper with Aβ aggregates and other molecular features of AD might also lead to decreased intracellular copper bioavailability and reduced activity of copper-dependent enzymes in the brain (16,17).

This “metal hypothesis of AD” is the basis of the development of treatments aimed at minimizing copper-induced neurotoxicity and restoring brain copper homeostasis (18). Preclinical and clinical studies demonstrated reduced Aβ burden, increased intracellular copper levels and improved cognitive function following administration of copper(II) chelators (e.g. Clioquinol, PBT2) capable of stripping copper ions from Aβ plaque and redistributing them into cells (19-21). A recent investigation showed that treatment with glyoxalbis(N4-methyl-3-thiosemicarbazonato) copper(II) (Cu-GTSM) - a copper bis(thiosemicarbazone) complex that delivers copper into cells and releases it bioreductively (22) - enhanced cognitive performance in APP/PS1 transgenic AD mice by triggering neuroprotective mechanisms that inhibited GSK3β and decreased phosphorylated tau and Aβ trimer levels (23). Hence, understanding the role of copper homeostasis in AD pathology may be critical to development of both novel diagnostics and therapeutics.

A PET radiotracer able to measure brain copper trafficking in vivo could be a valuable tool to investigate changes in copper metabolism in brain, to determine the potential of copper dyshomeostasis as an early diagnostic marker for AD and as a useful complement to other imaging biomarkers. Positron-emitting copper isotopes (60Cu, 61Cu,
$^{62}$Cu, $^{64}$Cu) can in principle be used to study the trafficking of copper to, from and within brain, not only in animal models but also in humans, by PET imaging over a period of hours and repeatedly during the lifetime of the subject. Intravenous injection of $^{64}$Cu in the form of ionic copper (II) salts leads to delivery of radiotracer to the brain via native copper transport processes in quantities that are insufficient for meaningful imaging (24, 25). However, by exploiting the ability of uncharged lipophilic copper(II) complexes, such as Cu-GTSM, to penetrate the blood-brain barrier (BBB) and release their copper payload non-selectively into cells (26), the intracellular copper pool can be supplemented with tracer quantities of $^{64}$Cu sufficient to allow imaging of its subsequent trafficking. A preliminary study by Fodero-Tavoletti and co-workers showed that 60 min after $^{64}$Cu-labeled Cu-GTSM was administered intravenously, brain uptake of $^{64}$Cu as measured by ex vivo gamma-counting was significantly greater in APP/PS1 mice than in their age-matched wild-type counterparts (27). Following up on this work, in the present study we explore further the trafficking of copper in AD by PET imaging with $^{64}$Cu-GTSM in the APPswe x PS1.M146V (TASTPM) transgenic mouse model. TASTPM mice rapidly develop some of the key pathological features of AD, such as cerebral Aβ plaque deposition, neuronal loss and neuroinflammation, showing impaired cognitive function at 6 months of age (28, 29). The visualization, mapping and monitoring of $^{64}$Cu trafficking in this model of AD will provide valuable information to determine the potential of imaging brain copper trafficking as a diagnostic marker for AD.

**MATERIALS AND METHODS**
Full experimental details for the radiosynthesis of $^{64}$Cu-GTSM (26, 30, 31), utilized animal models, solvent extraction method for determination of $^{64}$Cu-GTSM in brain tissue, nanoPET imaging (32), PET image analysis (33), texture analysis (34, 35), ex vivo biodistribution, brain autoradiography, histology, and statistical analysis are reported in the supplementary information. Animal experiments were performed in accordance with the Animals (Scientific Procedures) Act, 1986 and approved by the local ethics committee.

RESULTS

Solvent extraction
To assess the ability of $^{64}$Cu-GTSM to release $^{64}$Cu intracellularly within the brain, we determined the amount of intact complex in brain homogenates of mice injected with $^{64}$Cu-GTSM using a previously reported octanol-extraction method (36). Almost all $^{64}$Cu partitioned in the aqueous/protein-bound in both TASTPM mice and age-matched controls; 3.81 ± 2.98 % of brain $^{64}$Cu radioactivity was associated with the octanol fraction at 30 min and 2.82 ± 0.14 % at 24 h after injection. Radio-TLC showed that even the octanol-extractable fraction contained no intact $^{64}$Cu-GTSM.

PET imaging
Whole-body copper fluxes in vivo in TASTPM mice (n = 4) were imaged with $^{64}$Cu-GTSM from 0-30 min and from 24-25 h after injection and compared to wild-type controls (n = 6). The general biodistribution of the tracer was similar for both animal
groups (Fig. 1). At 30 min, $^{64}\text{Cu}$ radioactivity accumulated primarily in the heart, lungs, liver, intestines, kidneys, spinal cord and brain in both TASTPM and wild-type mice. After 24 h, a notable decrease in $^{64}\text{Cu}$ activity was observed in the lungs, kidneys and heart, whereas $^{64}\text{Cu}$ concentration in the brain declined much less (supplemental, Fig. S1).

$^{64}\text{Cu}$ biokinetics within the central nervous system (CNS: brain and spinal cord) were compared in AD mice and healthy controls using PET ROI analysis (see Fig. 2 and Fig. S2). PET quantification revealed that global average brain concentration of $^{64}\text{Cu}$ was significantly higher in TASTPM transgenic mice than in wild-type controls at 30 min ($7.83 \pm 1.08 \%\text{ID/g vs. 5.57 }\pm 0.70 \%\text{ID/g, } p<0.01$) and 24 h ($7.04 \pm 0.94 \%\text{ID/g vs. 5.46 }\pm 0.70 \%\text{ID/g, } p<0.05$). After 24 h, a significant decrease in brain $^{64}\text{Cu}$ concentration was found in TASTPM mice (-10.10 $\pm 2.23 \%$, $p<0.01$) but not in age-matched controls (-2.04 $\pm 3.57 \%$, $p>0.05$). TASTPM mice also exhibited significantly higher $^{64}\text{Cu}$ concentration in the spinal cord than controls at both 30 min ($5.20 \pm 0.67 \%\text{ID/g vs. 3.73 }\pm 0.54 \%\text{ID/g, } p<0.01$) and 24 h ($4.65 \pm 0.46 \%\text{ID/g vs. 3.48 }\pm 0.46 \%\text{ID/g, } p<0.05$).

**Atlas-based image analysis of regional brain concentration of $^{64}\text{Cu}$**

As depicted in Fig.3 (see also supplementary information, Fig. S3), $^{64}\text{Cu}$-GTSM distribution in the brains of both wild-type and TASTPM mice was heterogeneous both at 30 min and 24 h. To quantify heterogeneity in a functional/anatomical context, brain PET images were analyzed using a 3D mouse brain atlas (Fig. 4). TASTPM mice showed higher $^{64}\text{Cu}$ concentration than controls in all defined regions, reaching
statistical significance in the medulla (p<0.05), cerebellum (p<0.01), midbrain (p<0.001), hippocampus (p<0.05), thalamus (p<0.01), hypothalamus (p<0.05), pallidum (p<0.05), olfactory bulb (p<0.001) and corpus callosum (p<0.05) at 30 min, and in the medulla (p<0.001), cerebellum (p<0.01), midbrain (p<0.05), hypothalamus (p<0.01) and olfactory bulb (p<0.05) and pons (p<0.05), at 24 h (Supplemental Table S1).

**Texture analysis**

Statistical data emerging from texture analysis are listed in supplementary data (Table S2). The TASTPM group showed increased standard deviation (p<0.05) and coefficient of variation (p<0.01) compared to controls, demonstrating a higher spread and non-uniformity in voxel intensity values. TASTPM brain images had also higher entropy (p<0.01) than those of wild-type mice, revealing an increased level of disorder in voxel intensity distribution. The energy of TASTPM brain images was lower (p<0.05) compared to that of control datasets, suggesting a broader histogram and spread of voxel intensity values. The TASTPM group also showed a lower kurtosis (p<0.01) than controls, which is indicative of a voxel intensity distribution less clustered around the mean and more spread, compared to the control group. Skewness was more negative (p<0.01) for controls than for TASTPM mice, indicating a greater spread for voxel intensity values lower than the median than for those higher than the median in wild-type datasets. All measures of central tendency of the datasets (mean, median, maximum, minimum, range and percentiles) were higher for the TASTPM group than for wild-type mice, which is indicative of increased tracer uptake in the brains of TASTPM mice compared to controls.
**Ex vivo biodistribution**

Following PET imaging, the *ex vivo* biodistribution of $^{64}$Cu at 24 h post-injection of $^{64}$Cu-GTSM was evaluated by gamma-counting of explanted organs (Fig. 5). Only lung tracer uptake was found to differ significantly between groups and was higher in TASTPM than controls ($21.30 \pm 5.39 \text{%ID/g vs. } 17.54 \pm 1.22 \text{%ID/g, } p<0.05$). Global brain uptake was consistently raised in TASTPM mice compared to age-matched controls, but by the tests employed the difference was not significant ($p>0.05$). The brain %ID/g values determined by *ex vivo* organ counting showed a moderately strong correlation ($r^2 = 0.61$) with whole-brain uptake measured by PET (see supplementary data, Fig. S4).

**Ex vivo brain autoradiography**

To evaluate further the heterogeneity in the brain distribution of $^{64}$Cu, sagittal brain slices of TASTPM ($n = 4$) and control mice ($n = 6$) sacrificed 24 h after $^{64}$Cu-GTSM injection were imaged using phosphor imaging autoradiography. *Ex vivo* autoradiographs (Fig. 6) confirmed heterogeneous $^{64}$Cu uptake within brain tissue and the localization of radioactive hotspots in the medulla, cerebellum, pons, midbrain, caudate putamen and olfactory bulb seen by PET imaging. Visual inspection of representative digital autoradiographs showed increased tracer uptake and greater heterogeneity in $^{64}$Cu brain distribution in TASTPM mice compared to controls.

**Histology**
To determine whether the regional brain distribution of $^{64}$Cu was related to Aβ plaque deposition, TASTPM brain slices were autoradiographed and subsequently stained with Congo red (Fig. 7). Although $^{64}$Cu was taken up by the entire brain, the areas with the most prominent radioactive hotspots (olfactory bulb, medulla, cerebellum, midbrain, pons and caudate putamen) did not correspond to the regions with highest Aβ plaque load (hippocampus, thalamus and cerebral cortex).

**DISCUSSION**

In this study, we aimed to explore brain copper trafficking in TASTPM mice and age-matched controls at the earliest stage at which cognitive impairment can be detected in this transgenic model (i.e. 6 months old) (28), by PET imaging using $^{64}$Cu-GTSM. The chosen route of administration of $^{64}$Cu-GTSM was intravenous injection, which does not mimic the gastrointestinal uptake and biodistribution of endogenous copper but ensures rapid delivery of the radiotracer into the brain. To confirm the ability of the tracer to release radiocopper intracellularly within the brain, we used solvent extraction to measure the amount of intact (octanol-soluble) $^{64}$Cu-GTSM present in brain homogenates, at different time-points after injection. The low $^{64}$Cu present in the octanol-extractable fraction, and the lack of $^{64}$Cu-GTSM complex detectable by radio-TLC of that fraction, indicated that $^{64}$Cu-GTSM was almost completely dissociated by 30 min. Combined with the PET imaging studies which showed remarkably high and rapid $^{64}$Cu-GTSM uptake in the brain, this is consistent with a process in which the lipophilicity of the $^{64}$Cu-GTSM complex facilitates its passage across the BBB and into cells where it dissociates, releasing $^{64}$Cu intracellularly, as previously suggested for this family of
compounds (22, 26, 37, 38). The speciation of the dissociated $^{64}$Cu in the intracellular copper pool remains unknown. $^{64}$Cu-GTSM PET imaging also indicated that clearance of radiocopper from the brain is considerably slower than from most other tissues (e.g. heart, kidneys, lungs), for reasons not yet clear; it may be that brain tissue has greater active sequestration of copper ions driven by increased metabolic needs associated with regular brain function, or less active efflux mechanisms.

PET imaging of copper trafficking with $^{64}$Cu-GTSM revealed significantly increased brain and spinal cord uptake of $^{64}$Cu in TASTPM mice at 30 min and 24 h, compared to healthy controls. Despite differences in the animal model, age range and methods used, these results agree with those reported by Fodero-Tavoletti et al. (27), who reported enhanced $^{64}$Cu brain concentration in the APP/PS1 mouse model of AD. Unlike PET, the increase in $^{64}$Cu brain uptake in TASTPM mice compared to controls measured by ex vivo gamma-counting at 24 h post-injection of the tracer did not reach statistical significance; ex vivo organ counting is subject to greater opportunities for non-systematic error (e.g. cross contamination between tissues during dissection, weighing errors, incomplete explantation) that may account for this discrepancy. This may also explain why gamma-counting measurements did not show a stronger linear correlation with PET quantitative data ($r^2 = 0.61$).

While $^{64}$Cu clearance from brain was slower than that from other tissues in both TASTPM and wild-type mice, washout between 30 min and 24 h was significantly faster, by a factor of 5, in TASTPM mice than in healthy controls (-10 % vs. -2 %).
Nevertheless, TASTPM mice still had significantly higher $^{64}\text{Cu}$ concentration in brain than controls both at 30 min and 24 h. These differences in brain copper trafficking between the two groups may be due to more active copper influx and efflux mechanisms in the TASTPM model, or different patterns of copper bioavailability to these processes. The molecular mechanisms underlying the increased uptake in TASTPM brains have yet to be elucidated; it may be driven by the regulation of total brain copper levels in an effort to maintain brain copper homeostasis and/or enhance copper-dependent antioxidant enzyme activity as a response to oxidative stress. On the other hand, faster brain clearance of $^{64}\text{Cu}$ in TASTPM transgenic mice may be caused by the overexpression of APP, for which a role in normal copper metabolism as an efflux transporter has been suggested (11).

Both PET and *ex vivo* autoradiography demonstrated that accumulation of $^{64}\text{Cu}$ within the brain at 30 min and 24 h was heterogeneous. Seeking to rationalize $^{64}\text{Cu}$ this heterogeneity, we measured $^{64}\text{Cu}$ brain regional uptake using a 3D mouse brain atlas and found it to be higher in all brain regions in TASTPM mice compared to controls, with differences between the two groups reaching statistical significance in specific areas at 30 min and 24 h post-injection of the tracer. It should be noted that many of these brain regions are very small compared to the limited spatial resolution of the PET/CT scanner. The heterogeneity in $^{64}\text{Cu}$ concentration will therefore inevitably have been underestimated by atlas-based quantification due to the partial volume effect; combined with the lack of flexibility of segmentation boundaries and the potential for imperfect
matching of atlas regions onto real brain images, these results must be interpreted cautiously.

To avoid subjectivity in interpreting regional variations in brain uptake of $^{64}$Cu because of these limitations, PET brain signal heterogeneity was alternatively measured by texture analysis, which interrogates global and regional patterns of image heterogeneity without an underlying biological or structural model. Statistical analysis of first-order texture parameters revealed a higher spread, asymmetry and non-uniformity of the voxel intensity distribution in TASTPM brain images, indicating a greater degree of image heterogeneity in the disease model compared to controls. While this does not offer a biological interpretation of differences in brain uptake of $^{64}$Cu between the two animal groups, it does offer a basis for parameterisation of imaging for clinical diagnostic purposes.

The sequestration of copper by extracellular Aβ plaque deposits is thought to contribute to brain copper dyshomeostasis in AD by reducing the intracellular bioavailability of copper ions ($6, 12, 13, 39, 40$). To determine the extent of correlation between the regional distribution of $^{64}$Cu in the brain and Aβ plaque deposition in TASTPM mice, brain sections were imaged by autoradiography and subsequently stained with the Aβ-binding dye Congo red. The brain regions with the highest density of Congo red-positive amyloid deposits (hippocampus, thalamus and cerebral cortex) showed the lowest accumulation of radioactivity among the brain regions observed in the autoradiographs of the same sections at 24 h post-injection. This lack of correlation between the main
radioactive hotspots and the regions of highest Aβ plaque load does not support the hypothesis that amyloid plaques serve as a sink for copper ions (12,13), at least acutely, although it does not preclude the possibility that copper may accumulate in amyloid plaques chronically over the lifetime of the animal. It also suggests that metabolic/pathological factors other than Aβ plaque deposition may influence acute brain distribution of $^{64}$Cu, consistent with the view that amyloidosis may be a localised indicator of global disease.

CONCLUSION

$^{64}$Cu-GTSM can effect efficient delivery and release of $^{64}$Cu into the brain, and has potential for PET imaging to study changes in copper ion trafficking associated with AD and possibly other neurological disorders. PET imaging with $^{64}$Cu-GTSM showed statistically significant differences in $^{64}$Cu global brain uptake and clearance, as well as in brain regional $^{64}$Cu concentration and PET image heterogeneity, between TASTPM transgenic mice and age-matched wild-type controls. These results support clinical evaluation of $^{64}$Cu-GTSM PET imaging as a tool to investigate alterations in copper trafficking that might have added value in the diagnosis of AD.

DISCLOSURES

None
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REFERENCES


**Fig. 1.** Exemplar sagittal and coronal PET/CT images of wild-type controls and TASTPM mice at 30 min and 24 h post-administration of $^{64}$Cu-GTSM. Organs are indicated by arrows: B = brain; Sp = spinal cord; H = heart; Lu = lungs; L = liver; K = kidneys; I = intestines.
**Fig. 2.** *In vivo* brain concentration of $^{64}$Cu (%ID/g) and corresponding time-activity curves, in TASTPM mice and wild-type controls measured by PET ROI analysis. Significant differences are indicated by * (p<0.05) and ** (p<0.01). Data are mean (n = 4 for TASTPM mice; n = 6 for controls) ± SD. Similar results were obtained for spinal cord (see supplementary data, Fig. S2).
Fig. 3. Representative PET/CT MIP images illustrating heterogeneous distribution of $^{64}$Cu in brains of wild-type and TASTPM mice at 30 min (top) and 24 h (bottom) post-injection of $^{64}$Cu-GTSM. Images of all TASTPM ($n = 4$) and wild-type ($n = 6$) mice are included in the supplementary data (Fig. S3).
**Fig. 4.** Regional brain distribution of $^{64}$Cu in TASTPM mice and controls at 30 min (top) and 24 h (bottom). Statistically significant differences are indicated by * (p<0.05), ** (p<0.01) and *** (p<0.001). Data are mean (n = 4 for TASTPM mice; n = 6 for controls) ± SD.
Fig. 5. Tissue biodistribution (ex vivo) of $^{64}$Cu in 6-8-month-old TASTPM mice and age-matched controls at 24 h post-injection of $^{64}$Cu-GTSM. Statistically significant differences ($p < 0.05$) from controls are indicated by *. Data are mean ($n = 4$ for TASTPM mice; $n = 6$ for controls) ± SD.
Fig. 6. Surface plots of brain autoradiographs from a 6-8-month-old TASTPM (left) and control (right) mouse sacrificed 24 h after administration of $^{64}$Cu-GTSM.
Fig. 7. Photomicrograph of sagittal brain section from a TASTPM mouse (top) and corresponding brain autoradiograph (bottom) 24 h post-injection of $^{64}\text{Cu}$-GTSM.

ME = medulla; CE = cerebellum; M = midbrain; P = pons; C1 = cerebral cortex; H = hippocampus; TH = thalamus; CP = caudate putamen; LV = lateral ventricle; C2 = cerebral cortex; ON = anterior olfactory nucleus; OB = olfactory bulb.
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