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Positron emission tomography for measurement of copper fluxes in live organisms

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Abstract

Copper is an essential nutrient for the physiology of live organisms, but excessive copper can be harmful. Copper radioisotopes are used for measurement of copper fluxes in live organisms using a radioactivity assay of body fluids or whole-body positron emission tomography (PET). Hybrid positron emission tomography–computed tomography (PET/CT) is a versatile tool for real-time measurement of copper fluxes combining the high sensitivity and quantification capability of PET and the superior spatial resolution of CT for anatomic localization of radioactive tracer activity. Kinetic analysis of copper metabolism in the liver and other extra-hepatic tissues of *Atp7b*^{−/−} knockout mice, a mouse model of Wilson’s disease, demonstrated the feasibility of measuring copper fluxes in live organisms with PET/CT using copper-64 chloride (⁶⁴CuCl₂) as a radioactive tracer (⁶⁴CuCl₂-PET/CT). ⁶⁴CuCl₂-PET/CT holds potential as a useful tool for diagnosis of inherited and acquired human copper metabolism disorders, and for monitoring the effects of copper-modulating therapy.

Keywords

copper metabolism; Wilson’s disease; Menkes disease; positron emission tomography; PET/CT; copper-64 chloride

Copper is an essential nutrient element required for normal development and physiology of live organisms; however, excess copper is harmful.^{1–7} Absorption, retention, and excretion of copper are tightly regulated to maintain copper homeostasis by a systemic balance of influx and efflux of copper ions under physiologic conditions.^{4,8,9} Wilson’s disease is caused by mutation of the *ATP7B* gene,^{10,11} which encodes a Cu⁺-transporting β polypeptide ATPase, and is characterized by hepatic and neurological disorders resulting from the toxicity of the accumulation of excess copper ions in the liver and brain tissues. In contrast, Menkes disease is caused by mutation of the *ATP7A* gene, which encodes a Cu⁺-transporting α polypeptide ATPase, and is characterized by neurological disorders resulting from cerebral copper deficiency due to reduced absorption of copper and subsequent reduction of copper influx to the brain.^{12–15} Significant progress has been made in

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Conflict of interests

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describing the role of various copper transporters and chaperones in the regulation of copper metabolism at the molecular and cellular levels.^{16–20} However, many mechanistic details of the systemic regulation of copper metabolism remain elusive, partly due to the lack of a reliable tool for real-time kinetic analysis of copper fluxes in live organisms. Development of a tool for non-invasive measurement of copper fluxes in live organisms is not only significant for studying the systemic regulation of copper metabolism, but also significant for studying the pathophysiology of Wilson's disease, Menkes disease, and other inherited and acquired human copper metabolism disorders.

Copper radioisotopes

Copper radioisotopes were previously used for the study of copper metabolism in live organisms, based upon the principle of radioactive tracers invented by radiochemist and Nobel laureate George Charles de Hevesy.²¹ The gastrointestinal absorption, distribution, and excretion of copper in small animals were tracked by assessing the radioactivity of body fluids or tissues after administration of radioactive copper as a tracer.^{22–28} Radioactive copper isotopes were also used for investigation of copper metabolism in normal human subjects,^{9,29,30} and the disturbance of copper homeostasis in Wilson's disease^{31–35} and other liver diseases,³⁶ where the radioactivity of body fluids was assessed. Whole-body copper fluxes in humans were measured using a nuclear scintillation scanner (Picker Magnascanner) and radioactive copper as a tracer.^{37,38} Increased accumulation of radioactive copper was visualized in the liver, but measurement of copper fluxes in extrahepatic tissues was prevented by the limited spatial resolution of the nuclear scintillation scanner available at that time. In 1975, Skromne-Kadlubik *et al.*³⁹ reported a human basal ganglia scan using a scintillation camera after oral administration of 1 mCi of ⁶⁴Cu complex as a radioactive tracer (details of the preparation and characterization of this ⁶⁴Cu complex were not described in the report). The basal ganglia were visualized in the brains of normal human volunteers but not in the brain of one patient with Wilson's disease. The findings from these early clinical studies suggested the feasibility of non-invasive assessment of copper metabolism in humans using a copper radioactive tracer. However, clinical use of copper radioactive tracers for studying whole-body copper metabolism in humans was likely hampered by the limited spatial resolution of primitive nuclear scintillation cameras at the time.

PET for measurement of copper fluxes

Positron emission tomography (PET) scanners manufactured now have higher sensitivity and much better spatial resolution compared to the nuclear scintillation cameras previously used for studying copper metabolism in humans.^{37–39} Multiple positron emitter copper radioisotopes, which include ⁶⁰Cu ($t_{1/2}$ 23.7 min, 93% β^+ , 7% EC), ⁶¹Cu ($t_{1/2}$ 3.32 h, 60% β^+ , 40% EC), ⁶²Cu ($t_{1/2}$ 9.74 min; 98% β^+ , 2% EC), and ⁶⁴Cu ($t_{1/2}$ 12.7 h, 17.4% β^+ , 43% EC, 39% β^-), were evaluated for the preparation of PET radiopharmaceuticals.^{40–44} Among these copper radioisotopes, ⁶⁴Cu is particularly suitable for the measurement of whole-body copper fluxes in view of its desirable $t_{1/2}$ time of 12.7 h, the feasible production of ⁶⁴Cu with a biomedical cyclotron,⁴⁵ and the delivery of ⁶⁴Cu from the production site to a laboratory or imaging facility at a distant location. The introduction of a small-animal PET

scanner^{46–48} revived research interest in preclinical investigation of inherited and acquired copper metabolism disorders using ⁶⁴Cu (Table 1). In 2005, Bissig *et al.*⁴⁹ measured whole-body copper flux in 15-week-old Long–Evans cinnamon (LEC) rats, a rat model of Wilson's disease with defects of Atp7b,^{50, 51} using a 16-module variant of quad-HIDAC tomography (Oxford Positron Systems, Weston-on-the-Green, United Kingdom) and ⁶⁴CuSO₄ as a radioactive tracer. Prolonged retention of ⁶⁴Cu was visualized in the gastrointestinal tracts of LEC rats after transgastric injection of the tracer ⁶⁴CuSO₄. Insufficient spatial resolution of the small-animal PET scanner still limited real-time kinetic analysis of low ⁶⁴Cu radioactivity in the liver and other extrahepatic tissues of the LEC rats. The authors reported the use of sampling and β -counting of the radioactivity of bile fluid to track the absorption and transportation of copper to the liver, and demonstrated a reduction of hepatobiliary excretion of ⁶⁴Cu in the LEC rats, compared to that in wild type normal control rats. In 2012, Bahde *et al.*⁵² reported the use of a small-animal PET scanner (R4 scanner, Concorde MicroSystems, Siemens Medical Solutions USA, Inc.) for measurement of whole-body copper fluxes in LEC rats and healthy Long–Evans agouti (LEA) rats, using ⁶⁴Cu–histidine complexes as a radioactive tracer. In the healthy LEA rats, there was rapid clearance from the blood, hepatic uptake, and hepatobiliary excretion of ⁶⁴Cu radioactivity. In contrast, ⁶⁴Cu was efficiently cleared from the blood with hepatic uptake, but ⁶⁴Cu was not excreted in the bile in LEC rats due to malfunction of the Atp7b copper transporter. More interestingly, PET dynamic analysis demonstrated partial restoration of hepatobiliary excretion of ⁶⁴Cu in LEC rats after transplantation of healthy LEA rat hepatocytes to the liver of LEC rats, suggesting reconstitution of Atp7b function in the liver of LEC rats following transplantation of healthy LEA hepatocytes. The data from this preclinical study suggested the potential use of PET for monitoring the therapeutic effects of hepatocyte transplantation for the treatment of WD. Again, copper fluxes in extrahepatic tissues were not measured, most likely owing to the limited spatial resolution of the small-animal PET scanner, which prevented the anatomic localization of low levels of copper radioactive tracer in extrahepatic tissues after injection of ⁶⁴Cu–histidine.

PET/CT for measurement of copper fluxes

A hybrid PET/CT scanner is a versatile tool combining the high sensitivity and quantification capability of PET and the superior spatial resolution of CT for anatomic localization of radioactive tracer activity.⁵³ Small-animal hybrid PET/CT scanners were developed for the preclinical characterization of PET radiopharmaceuticals and the functional imaging of various metabolic pathways using an animal model of human disease. In 2012, Peng *et al.*⁵⁴ reported non-invasive measurement of whole-body copper fluxes in a live organism using a small-animal PET/CT scanner (Inveon PET/CT Multimodality System, Siemens, St. Louis, Missouri). Aided by the high spatial resolution of CT, the investigators were able to measure whole-body copper fluxes in *Atp7b*^{−/−} knockout mice, an established mouse model of human WD.^{55,56} Based on the previous use of ⁶⁴CuCl₂ in humans, ⁶⁴Cu in the form of ⁶⁴CuCl₂ in 0.1N HCl (Washington University, St Louis, Missouri) was diluted with normal saline containing 0.9% NaCl and injected intravenously into the tail vein. Immediately after administration of the radioactive tracer (⁶⁴CuCl₂ at a dose of 74 kBq or 2 μ Ci/g body weight), dynamic whole-body data acquisition proceeded

for one hour, followed by static acquisition for 15 minutes at 2 h and 24 h after injection of the tracer. Time-activity curves from real-time kinetic analysis demonstrated a continued increase of ^{64}Cu in the liver of *Atp7b*^{-/-} mice, in contrast to initial rapid uptake and a gradual clearance of ^{64}Cu from the liver to the intestines via the hepatobiliary clearance pathway in the control C57BL/6 mice. Renal uptake of ^{64}Cu in the *Atp7b*^{-/-} knockout mice in the initial 60 minutes was similar to that of the control C57BL mice. Although ^{64}Cu radioactivity in both the heart and lungs of *Atp7b*^{-/-} mice was higher than that of control C57BL/6 mice, ^{64}Cu radioactivity in the heart and lungs of *Atp7b*^{-/-} knockout mice was found to be lower than that in control C57BL/6 mice at 24 h after injection of the tracer. Surprisingly, quantitative analysis revealed consistently lower ^{64}Cu radioactivity in the brain of *Atp7b*^{-/-} mice compared to that in C57BL/6 mice. It was postulated that the reduced ^{64}Cu radioactivity in the brain and other extrahepatic tissues of *Atp7b*^{-/-} mice might be caused by reduced bioavailability of copper owing to hepatic copper sequestration.

To assess the gastrointestinal uptake and biodistribution of dietary copper under physiological and pathophysiological conditions, the investigators also measured whole-body copper fluxes in *Atp7b*^{-/-} mice with PET/CT after oral administration of $^{64}\text{CuCl}_2$ as a radioactive tracer using a Siemens Inveon PET/CT Multimodality System.⁵⁷ Prolonged trapping of abundant ^{64}Cu radioactivity in the gastrointestinal tract was visualized in the *Atp7b*^{-/-} mice orally administered $^{64}\text{CuCl}_2$, which was in agreement with the findings reported previously by Bissig *et al.*⁴⁹ Time-activity curves from dynamic PET quantitative analysis showed initial higher hepatic uptake of ^{64}Cu absorbed from the gastrointestinal tract in *Atp7b*^{-/-} mice than that in C57BL/6 WT mice following oral administration of $^{64}\text{CuCl}_2$. There was higher ^{64}Cu radioactivity in the kidneys of *Atp7b*^{-/-} mice compared to that in the kidneys of control C57BL/6 mice, indicating more urine clearance of ^{64}Cu in *Atp7b*^{-/-} mice than that in control C57BL/6 mice. However, there was no significant difference in the low ^{64}Cu radioactivity of the blood, brain, heart, and muscles between the 12- to 13-week-old *Atp7b*^{-/-} mice and age-matched control C57BL/6 mice. The ^{64}Cu radioactivity measured by PET/CT represents the sum of the influx and efflux of ^{64}Cu . The results of increased hepatic ^{64}Cu radioactivity detected by PET/CT correlated well with the previous demonstration of increased copper ions in the liver of *Atp7b*^{-/-} mice.⁵⁵ This suggests that $^{64}\text{CuCl}_2$ -PET/CT may be used to quantify copper ions, assuming that ^{64}Cu radioactivity detected by PET/CT reflects the amount of copper ions accumulated in the organ or tissues. This needs to be validated by a direct comparison of ^{64}Cu radioactivity measured by PET/CT with the amount of copper ions quantified by other methods.⁵⁸⁻⁶²

Human radiation dosimetry estimates of $^{64}\text{CuCl}_2$ were obtained in these preclinical studies, based on the biodistribution of ^{64}Cu radioactivity in *Atp7b*^{-/-} mice and control C57BL/6 mice.^{54,57} Human radiation dosimetry estimates of intravenously injected $^{64}\text{CuCl}_2$ were predicted to be comparable to that of F-18 FDG,⁶³ when 5 MBq/kg (0.14 mCi/kg) of $^{64}\text{CuCl}_2$ was administered for PET in normal human subjects and 4 MBq/kg (0.11 mCi/kg) of $^{64}\text{CuCl}_2$ is administered for PET in a patient suspected of WD.⁵⁴ When 5 MBq/kg (0.14 mCi/kg) of $^{64}\text{CuCl}_2$ was orally administered, effective radiation dose was about 30% higher than that in 2-deoxy-2-[^{18}F]-fluoro-*D*-glucose [^{18}F]-FDG PET studies.⁶³ However, the average radiation dose to the critical organ (lower large intestines)

from $^{64}\text{CuCl}_2$ was about half that of [^{18}F]-FDG to its critical organ (urinary bladder) for PET in humans. This suggests the overall radiation dose for a PET study using orally administered $^{64}\text{CuCl}_2$ is comparable to that for the established [^{18}F]-FDG PET. Nevertheless, this needs to be corrected in clinical radiation dosimetry of $^{64}\text{CuCl}_2$ in view of the potential difference in copper metabolism between rodents and humans.

Furthermore, a longitudinal PET/CT scan with orally administered $^{64}\text{CuCl}_2$ was used for quantitative analysis of whole-body copper fluxes in a study of age-dependant changes of hepatic and renal clearance of copper in the *Atp7b*^{-/-} mice.⁶⁴ The investigators were able to demonstrate age-dependent changes of copper fluxes in the liver and other organs in the *Atp7b*^{-/-} mice by PET/CT after oral administration of the radioactive tracer $^{64}\text{CuCl}_2$. Hepatic ^{64}Cu radioactivity in the *Atp7b*^{-/-} mice at 13 and 20 weeks of age was lower than that in the *Atp7b*^{-/-} mice at 6 weeks of age at 24 h after oral administration of $^{64}\text{CuCl}_2$, suggesting an age-dependent decrease in hepatic accumulation of copper absorbed from diet. Age-dependent reduction of hepatic ^{64}Cu radioactivity was found to coincide with an increase in ^{64}Cu radioactivity in the kidneys and urinary bladder, in agreement with the previous observation of increased urinary excretion of copper in patients diagnosed with WD.^{65,66} This provided further evidence to support the use of $^{64}\text{CuCl}_2$ -PET/CT for measurement of copper fluxes in humans.

Perspectives

Initial use of $^{64}\text{CuCl}_2$ -PET/CT for investigation of copper metabolism imbalance in the *Atp7b*^{-/-} mice^{54,57,64} invites additional studies using PET/CT for noninvasive measurement of copper fluxes in live organisms. Although $^{64}\text{CuCl}_2$ was previously used for studying copper metabolism in humans,^{9,29-39} no ^{64}Cu radiopharmaceuticals are commercially available for non-invasive measurement of copper fluxes in humans using PET or PET/CT at this time. In view of the fact that capsules containing ^{123}I - or ^{131}I -NaI are routinely administered orally as a radiopharmaceutical for diagnosis and treatment of thyroid disease in the nuclear medicine clinic, oral administration of $^{64}\text{CuCl}_2$ appears to be a desirable feature of this potentially useful radiopharmaceutical for diagnosis and monitoring treatment of human copper metabolism disorders. Genetic testing alone may not be sufficient for diagnostic testing of WD because patients with identical mutations may show very different clinical phenotypes, such as the time of onset, variation and severity of symptoms, and progression of disease.⁶⁵⁻⁶⁷ Noninvasive real-time assessment of whole-body copper fluxes using $^{64}\text{CuCl}_2$ -PET/CT may shed new light on the pathophysiology of WD and other human copper metabolism disorders. From the clinic point of view, $^{64}\text{CuCl}_2$ -PET/CT holds potential as a diagnostic imaging test for early diagnosis of WD⁶⁵⁻⁶⁸ and as a tool for monitoring the effects of personalized copper-modulating therapy for WD.

In addition to inherited copper metabolism disorders, $^{64}\text{CuCl}_2$ -PET/CT may also be a useful tool for the investigation of acquired copper metabolism disorders, for example, neurological disorders related to acquired copper deficiency.⁶⁹⁻⁷⁷ Because the symptoms associated with copper deficiency are often variable and nonspecific, $^{64}\text{CuCl}_2$ -PET/CT may be used to measure copper fluxes in patients suspected of acquired copper deficiency in order to determine whether neurological symptoms are secondary to cerebral copper

deficiency caused by abnormal absorption of dietary copper. An emerging body of evidence suggests a role for altered copper metabolism in the pathogenesis of Alzheimer's disease,^{78–85} Parkinson's disease,^{59,82,85} and Huntington's disease.⁸⁶ Based on its demonstrated feasibility for measuring copper fluxes in live organisms, ⁶⁴CuCl₂-PET/CT holds potential as a valuable tool for investigating cerebral copper metabolism disturbance in patients suspected of neurodegenerative disorders. Copper is required for cell proliferation and angiogenesis, and alteration of copper metabolism may play a role in carcinogenesis.^{87–89} Previously, tumors (extrahepatic hepatoma, human prostate cancer xenografts, and human hepatocellular carcinoma xenografts) with increased uptake of ⁶⁴Cu in mice were visualized on PET images using a small-animal PET scanner (microPET R4 tomograph, Concorde MicroSystems) after intravenous injection of ⁶⁴CuCl₂ as a radioactive tracer.^{90–92} More recently, increased uptake of ⁶⁴Cu by human tumor xenografts was detected in mice injected with ⁶⁴CuCl₂ by PET, using a small-animal PET scanner in conjunction with the use of a small-animal CT scanner.⁹³ Targeting altered copper metabolism in cancer, ⁶⁴CuCl₂-PET/CT holds potential as a tool for diagnostic imaging and personalized therapy of copper hypermetabolic cancers.

Distinct advantages of using ⁶⁴CuCl₂-PET/CT for noninvasive measurement of copper fluxes in live organisms include its high sensitivity, quantification capability, and improved spatial resolution compared to ⁶⁴CuCl₂-PET. On the other hand, ⁶⁴CuCl₂-PET/CT has some limitations, which include its high cost; limited use for measurement of copper fluxes within a small, subcentimeter area of tissues or organs; and exposure to radiation, although the level of exposure to radiation from ⁶⁴CuCl₂-PET is low and considered safe. MRI is an attractive imaging modality for the measurement of copper fluxes in live organisms, particularly measurement of copper fluxes in brains, in view of its superb spatial resolution and the lack of exposure to radiation. Attempts were made to develop copper-responsive chemical agents for the quantification of copper ions in animal organs or tissues by MRI.^{94,95} It remains to be determined whether it is feasible to track copper fluxes in live organisms with MRI using these copper-responsive agents in view of the low sensitivity of MRI relative to the high sensitivity of PET. Optical imaging is another promising technique for measurement of copper fluxes in live organisms, particularly preclinical studies using small animals, in view of its high sensitivity, convenience, and low cost. Multiple copper-responsive fluorescent tracers were developed for measurement of copper flow at the cellular level.^{96–98} The application of fluorescent tracers for measurement of copper fluxes in humans by optical imaging needs to overcome limited tissue penetration, high background fluorescence, and quenching of fluorescence signals. It was reported that a near-infrared fluorescent sensor was successfully used as a tracer for measurement of copper flow in a murine model of WD.⁶¹ Continued search for additional tracers will in no doubt facilitate the development of new technologies allowing quantitative and longitudinal measurement of copper fluxes *in vivo*, providing a better understanding of the physiology of copper metabolism and the pathophysiology of inherited and acquired copper metabolism disorders in humans.

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Table 1

Preclinical use of PET or PET/CT for measurement of copper fluxes in live organisms

Year	PET Scanner	Tracer	Route of tracer administration	Subject	Reference
2005	quad-HIDAC tomography	$^{64}\text{CuSO}_4$	Transgastric injection	LEC rats ^a	49
2005	microPET R4 tomograph	$^{64}\text{CuCl}_2$	I.V.	Mice bearing extrahepatic hepatoma	90
2006	microPET R4 tomograph	$^{64}\text{CuCl}_2$	I.V.	Mice bearing human prostate cancer xenografts	91
2011	microPET R4 tomograph	$^{64}\text{CuCl}_2$	I.V.	Mice bearing human hepatoma xenografts	92
2012	Inveon PET/CT multimodality System	$^{64}\text{CuCl}_2$	I.V.	<i>Atp7b</i> ^{-/-} mice ^b	54
2012	R4 scanner, Concorde MicroSystems	^{64}Cu - histidine	I.V. or intrasplenic injection	LEC rats	52
2012	Inveon PET/CT multimodality System	$^{64}\text{CuCl}_2$	P.O.	<i>Atp7b</i> ^{-/-} mice	57
2012	Inveon PET/CT multimodality System	$^{64}\text{CuCl}_2$	P.O.	<i>Atp7b</i> ^{-/-} mice	64
2013	microPET Focus 120	$^{64}\text{CuCl}_2$	I.V.	Mice bearing human tumor xenografts	93

^a LEC rat, Long-Evans cinnamon rat model of Wilson's disease
^b *Atp7b*^{-/-} mice, *Atp7b*^{-/-} knockout mouse model of Wilson's disease
I.V., intravenous; P.O., per oral.