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Visualization of Copper Metabolism by $^{64}CuCl_2$-PET

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Copper plays an essential role in human physiology. It is required for respiration, radical defense, neuronal myelination, angiogenesis, and many other processes [1]. At the molecular level, copper metabolism and iron metabolism are closely linked. Disruption of ATPase Cu^{2+} transporting beta peptide (Atp7b) leads to relevant changes in iron homeostasis in addition to the known changes in copper metabolism [2]. Studies in humans of dietary copper intake, copper excretion, and copper balance, and studies in cadavers of tissue copper concentrations were reported in the 1930s and 1940s. However, many challenges exist with regard to whole-body copper metabolism study in humans [3]. In contrast with studies in laboratory animals, few tissues are available for analysis, limiting what can be learned directly from human studies. The amount of dietary minerals and the mineral content of blood and excretion products can be measured readily in human studies. Other tissues such as skin and liver can be sampled, but the sampling procedures are considered invasive. Concentrations of minerals in tissues and excreta provide limited information on their metabolism [3].

The introduction of isotopic tracers and kinetics modeling adds a new dimension beyond what can be learned in humans by direct measurement. Two stable isotopes of copper, ^{63}Cu and ^{65}Cu, have been proven to be very effective in providing information related to copper absorption, bioavailability, and excretion patterns. The primary advantages of the stable isotopes of copper are that they can be used as tracers with no exposure to radioactivity and that they do not decay. However, these two stable isotopes are relatively abundant which is not ideal for metabolic studies because the experimental ^{63}Cu or ^{65}Cu intake cannot be separated in collected samples from what is consumed through meals and from endogenous mineral. Besides, methods for measuring stable isotopes require specialized, costly equipment. Sample preparation is extensive, analysis is slow, and the isotopes are expensive [4, 5].

Two copper radioisotopes, ^{64}Cu ($t_{1/2}$ =12.8 h) and ^{67}Cu ($t_{1/2}$=58.5 h) have been used in metabolic research [6]. Research on copper balance in humans using copper radioisotopes began in 1947 [7], in which investigators followed the fate of radioactive copper in the blood after intravenous and subcutaneous injections [7]. Osborn *et al*. [8] were the first to describe a quantitative method to follow the sequestration of copper by the liver. However, their technique had several inherent weaknesses. First, it was necessary to estimate the liver weight from a formula which took no account of the possible effect of liver damage; second, it was necessary to estimate the thickness of tissue overlaying the liver in order to allow for scatter and absorption; third, the detector had to be accurately aligned to see the maximum amount of liver tissue at each determination since small variations in positioning would

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result in considerable differences in the count rate recorded; and finally, limited spatial resolution of the scintiscanner available at that time did not allow more detailed and quantitative analysis of copper radioactivity at various tissues and organs. Despite these shortcomings, they were able to demonstrate that the rate of the uptake of copper by the liver of patients with Wilsons's disease varies with the state of the illness.

Recent advances in hybrid positron emission tomography and X-ray computed tomography (PET-CT) makes it possible to noninvasively analyze copper metabolism in extrahepatic tissue in a real-time manner. In this issue of *Molecular Imaging and Biology*, Peng *et al*. [9] reported a novel application of PET-CT for copper metabolism analysis. Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism characterized by copper accumulation in various tissues, mainly in the liver and brain [10–12]. *Atp7b*$^{-/-}$ mice, a mouse model of human WD, were injected with $^{64}CuCl_2$ intravenously and underwent PET scanning using a hybrid small animal PET-CT scanner. Dynamic PET analysis revealed increased accumulation and markedly reduced clearance of ^{64}Cu from the liver of *Atp7b*$^{-/-}$ mice. In contrast to the liver, ^{64}Cu radioactivity from extrahepatic tissues, including brain, kidneys, lungs, and heart, is significantly lower in the *Atp7b*$^{-/-}$ mice compared to normal mice, which may be caused by the different hepatic sequestration patterns of ^{64}Cu in these two groups. This is a first study reporting the direct (or real-time) visualization and measurement of ^{64}Cu accumulation in extrahepatic organs in living organism.

Copper has several radioisotopes [6], among which ^{64}Cu has a relatively long half-life, can be conveniently produced by a biomedical cyclotron, and is commercially available in a ready-to-use chemical form. However, using ^{64}Cu radioisotope is associated with exposure to radioactivity. To address the safety of $^{64}CuCl_2$ PET, Peng *et al*. calculated radiation dosimetry of ^{64}Cu radioactivity. Based on the $^{64}CuCl_2$ biodistribution in *Atp7b*$^{-/-}$ mice, the effective dose was estimated to be 32.8 μSv/MBq, with the liver as critical organ for radiation dose (120 μSv/MBq for male adults and 161 μSv/MBq for female adults). This radiation dosimetry estimation encourages further evaluation of utilizing $^{64}CuCl_2$ as a tracer for noninvasive assessment of copper metabolism in WD with PET.

Several conditions and diseases influence whole-body copper metabolism. The conditions include inherited copper metabolic defects, such as Menkes syndrome and Wilson disease [13] and acquired copper metabolism disorder or imbalance caused by pregnancy [13], inflammation [14], and tumor growth, metastasis, angiogenesis as well as drug resistance [15–18]. Visualization of copper metabolism by PET with $^{64}CuCl_2$ may have applications in these conditions. First, it may change the management of WD. For example, some WD patients have copper accumulation in the brain and show neurological disorder while others do not. It was assumed that WD patient will have increased uptake of copper in brain tissue and pathologic analysis of the brain may show gliosis and neuronal loss in association with increased Cu deposition [19]. However, in Peng's study, they observed decreased ^{64}Cu uptake in brain tissue of *Atp7b*$^{-/-}$ knockout mice as compared with that of normal mice. This finding is novel and suggests that $^{64}CuCl_2$-PET could reflect the symptoms variation of WD and thus affect the treatment strategy. In addition, diagnosis of Wilson's disease can be challenging as symptoms mimic other diseases and may gradually appear over time. The presence of many different mutations in *Atp7b* gene makes it difficult to screen and diagnose WD by genetic testing. Symptoms evaluation along with blood test, urine test, eye test, brain CT or MRI scan, liver biopsy, or genetic testing are needed to make a diagnosis. With more detailed information and an SUV cutoff value obtained from further validation of the methodology reported here, $^{64}CuCl_2$-PET could serve as a simple, straightforward, and noninvasive method for WD diagnosis. Second, copper metabolism in cancer patients varies from individual to individual. $^{64}CuCl_2$-PET can be applied to monitor copper metabolism status and guide personalized copper chelator treatment in cancer patients [20]. Finally,

metal-labeled organic molecules, peptides, and antibodies have been widely used for diagnosis and therapy of many disease types. However, due to the fact that the metal may leak out of the chelate, these tracers do not necessarily fully reflect the behavior of the macrocycle conjugates. By comparing the *in vivo* kinetics of $^{64}CuCl_2$ and ^{64}Cu chelators, we can evaluate the stability of ^{64}Cu chelators before the chelator is used for ligand conjugation.

In conclusion, the results from this paper shed new light on elucidation of copper metabolism with noninvasive and quantitative $^{64}CuCl_2$-PET. Many questions remain to be answered in the field of copper metabolism. With new techniques and tools available, including radioisotope tracers, stable isotope tracers, and compartmental modeling kinetic analysis, investigators are gaining a better understanding of whole-body copper metabolism in humans.

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