Application of a Small Molecule Radiopharmaceutical Concept to Improve Kinetics

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Abstract Recently, large molecules or nanoparticles are actively studied as radiopharmaceuticals. However, their kinetics is problematic because of a slow penetration through the capillaries and slow distribution to the target. To improve the kinetics, a two-step targeting method can be applied by using small molecules and very rapid copper-free click reaction. Although this method might have limitations such as internalization of the first targeted conjugate, it will provide high target-to-non-target ratio imaging of radiopharmaceuticals.

The majority of radiopharmaceuticals belong to small molecules of which the molecular weight is less than 2000 Da, and the molecular size is smaller than 2 nm generally. The outstanding feature of the small molecule radiopharmaceuticals compared to large molecules is with their kinetics. Their distribution to target and clearance from non-target tissues are very rapid, which is the essential requirement of radiopharmaceuticals.

The penetration of these small radiopharmaceuticals through the cell membrane mainly depends on the lipophilicity. Lipophilic radiopharmaceuticals can penetrate cell membranes rapidly and show rapid tissue distribution. For example, [¹²³I]IMP, ^{99m}Tc-HMPAO, and ^{99m}Tc-ECD are very

lipophilic agents and thus penetrate cell membranes including the blood–brain barrier (BBB) very quickly [1–4]. The penetrated agents are trapped in the brain cells by various mechanisms. The final aspects of these lipophilic agents trapped in the brain represent cerebral blood flow due to their very rapid tissue uptakes.

However, hydrophilic radiopharmaceuticals should pass through the intercellular gaps between vascular endothelial cells of the capillary to reach to the target cells, which is generally much slower than passive diffusion through the cell membrane. Specific carriers or transporters are required for transporting hydrophilic radiopharmaceuticals into the cells. For example, glucose transporters, amino acid transporters, and Na⁺K⁺-ATPase are required for the uptake of [¹⁸F]FDG, [¹¹C]methionine, and ²⁰¹Tl, respectively [5–7]. Among these, the uptake rates of [¹⁸F]FDG and [¹¹C]methionine are much slower than ²⁰¹Tl. Thus, the uptake patterns of [¹⁸F]FDG and [¹¹C]methionine do not represent tissue blood flows, but glucose and amino acid uptake rates, respectively. However, uptake of ²⁰¹Tl through the Na⁺K⁺-ATPase is very rapid and represents myocardial blood flow rather than the activity of Na⁺K⁺-ATPase.

A review article, “In vivo evaluation of ¹⁸F-labeled mitochondrial voltage sensors as myocardial imaging agent using positron emission tomography,” written by Kim and Min is going to be published. The fluoroalkylphosphonium derivatives including (5-[¹⁸F]fluoropentyl)triphenylphosphonium cation (¹⁸F-FPTP), (6-[¹⁸F]fluorohexyl) triphenylphosphonium cation ([¹⁸F]FHTP), and (2-(2-[¹⁸F]fluoroethoxy)ethyl) triphenylphosphonium cation ([¹⁸F]FETP) are lipophilic cations that accumulate in mitochondria after passive diffusion, such as ^{99m}Tc-sestaMIBI (Fig. 1) [8]. They show very fast myocardial uptake and thus represent myocardial blood flow.

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Fig. 1 Chemical structures of lipophilic cation radiopharmaceuticals

However, the efficiency of radioisotope delivery to target by small molecule radiopharmaceuticals is low due to rapid renal excretion.

Various large molecules or nanoparticles have advantages of multimodality targeting or more efficient delivery of drugs or radioisotopes. However, unlike the small molecule radiopharmaceuticals, macromolecule radiopharmaceuticals, such as radiolabeled antibodies, show slow uptake to targets and sustained activity in the blood pool. They are not permeable to biological membranes and thus pass through the intercellular gaps of the capillaries to reach to the target. Also, this slow penetration from large molecular size caused slow *in vivo* target uptake. Blood pool activity decrease slowly because they are not excreted through the kidneys. To solve these problems, the advantage of small molecule radiopharmaceuticals was considered, which appeared as a two-step targeting strategy.

The early form of a two-step targeting strategy was the application of the avidin-biotin system, which has one of the strongest binding affinities among the existing biological systems [9]. A two-step targeting method would be performed by an administration of antibody-avidin conjugate, followed by radiolabeled biotin. After the first administration, the conjugate is distributed to target slowly. If the radiolabeled biotin is administered after a long enough distribution period, it distributes to the target fast, and the unbound fraction is excreted quickly due to its small molecular size. Although this method showed significantly improved targeting and attracted many researchers, it could not be applied widely due to several problems. The conjugate of antibody and avidin can decrease

the immunoreactive fraction of the antibody due to denaturation after conjugation, which significantly decreases the uptake to the target [10]. Moreover, the conjugate has a higher molecular weight than the antibody itself, which makes it more difficult to pass through the blood capillary. Another problem with this two-step targeting method is the possibility of internalization of antibodies. It is well-known that many antibodies are internalized after binding on the surface of cells, which restricts the application of the two-step targeting system because the labeled biotin molecule is administered in the time gap of the antibody-avidin conjugate binding to cancer cell and the internalization into the cancer cell.

Recently, a bio-orthogonal two-step targeting method was developed by application of *in vivo* copper-free click reaction (Fig. 2) [11, 12]. This method has advantages over the previous avidin-biotin system. The chemicals used for the copper-free click reaction are small molecules, unlike avidin, and thus the molecular size of the conjugated antibody or nanoparticle does not increase significantly. Although the avidin-biotin conjugation reaction is fast, the click reaction might be faster. In addition, there is no intrinsic interfering molecule, such as biotin. However, the problem of antibody internalization still exists. A review article, “Click reaction: an applicable radiolabeling method for molecular imaging,” written by Choi and Lee is going to be published.

In conclusion, the small molecule radiopharmaceuticals generally show excellent biodistribution properties; however, they show poor efficiency of radioisotope delivery. Large molecule or nanoparticle radiopharmaceuticals have advantages of multimodal and efficient delivery, but lower target-to-non-

Fig. 2 Conjugation of R and R' by using a copper-free click chemistry

target ratio. Two-step targeting using a bio-orthogonal copper-free click reaction can be a solution of the problem of large molecule or nanoparticle radiopharmaceuticals.

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