Use of ^{11}C-acetate PET imaging in the evaluation of advanced atherogenic lesions

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The use of ^{11}C-acetate as a PET/CT tracer for atherosclerotic lesions preferentially labels anti-inflammatory/pro-resolution intra-plaque macrophages. An overview of the mechanisms involved in the selective uptake.

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One of the areas of current interest in the field of evaluation of atherogenic lesions is the use of imaging techniques, in an attempt to determine both the size of the atheromatous plaque and the information related to its stability.[1-3] Indeed, unstable atheromatous lesions are the result of continuous adverse remodeling of the plaque, generally associated with a recruitment of pro-inflammatory immune cells. These complex mechanisms ends up generating serious atherothrombotic events, such as stroke or transient ischemic attacks. In fact, the serum biomarkers commonly used to determine atherogenic plaques at risk are quite limited and their predictive capacity is modest. Many of the severe atherothrombotic accidents are due to non-culprit major adverse cardiovascular events.[4,5] For this reason, molecular imaging approaches are of the outmost importance to establish the characteristics of the cells that make up the atherogenic nucleus: On the one hand, stable plaques constitute a relatively low-risk situation facing atheroma rupture. These plaques usually have a nucleus with a high content of necrotic cells, loaded with lipids, whose main adverse consequence is to alter the laminar regimen in the blood flow circulation. However, there are recurrent events that involve the entry of immunocompetent cells into the cap, which contribute to the generation of an active intraplaque inflammatory response. These cells, including monocytes/macrophages, can express extracellular matrix metalloproteinases, leading to remodeling of the atherosclerotic lesion. In some cases, the consequence of this remodeling is that the plaque becomes destabilized, anticipating the appearance of atherothrombotic events, the severity of which will depend on the size of the atheroma released and its location. Since this proteolytic remodeling capacity depends mainly on the pro-inflammatory nature of macrophages, the discrimination between pro-inflammatory (generally simplified as M1 macrophages) *vs.* anti-inflammatory/pro-resolving macrophages (M2 subtypes macrophages) within the atheromatous plaque is of relevance.[6-8] Interestingly, heterogeneity between these different macrophage phenotypes coexists in the atherogenic lesion.[9,10] Here, in the work by Demirdelen et al. [11] the authors have used ^{11}C-acetate-PET based imaging to assess the nature and profile of the cells present in atherosclerotic lesions. The group used an ApoE-deficient mice model of advanced atherogenesis, after feeding a western-type diet for 33 weeks. Under these conditions, the main active atheromatous lesions are located in the brachiocephalic arteries, an anatomical region reminiscent of the advanced human atherogenesis. In this model, the authors demonstrate, using both *in vivo* and *ex vivo* experiments that M2 polarized macrophages, rather than pro-inflammatory M1 macrophages, efficiently incorporate acetate (both ^{11}C-acetate in PET imaging, and ^{14}C-acetate in metabolic studies). Other cells present in the

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atheroma, such as vascular smooth muscle cells, contribute minimally to acetate uptake. According to their data, this is because M2 macrophages retain a significant activity in the TCA cycle, fueling acetate through the lipogenic pathway, as well as showing an enhanced mitochondrial biogenesis (favored by acetate itself). The use of acetate as a tracer in PET and MRI studies has been previously described.[2,12] In addition, as mentioned by the authors, ^{11}C-acetate PET/CT imaging offers significant advantages over the poor resolution of FDG techniques when imaging atherogenesis. For this reason, other alternatives have been envisaged, including more efficient substrates to be used by macrophages, such as ^{18}F-2-deoxy-manose,[13] or increasing the glycolytic flux of the atherogenic macrophages after priming with various cytokines, like GM-CSF or G-CSF.[14,15]

The uptake, biosynthesis and metabolism of acetate by mammalian cells is a topic of current interest. Recently, a *de novo* pathway of acetate biosynthesis from pyruvate has been described, in addition to the classic origin from ethanol or from the colonic fermentation by the microbiota.[16,17] Indeed, acetate has been identified as an appetite suppressant in the central nervous system.[17] In macrophages, acetate is metabolized mainly by Acetyl-CoA synthetase (ACS), an enzyme highly expressed in M1 macrophages and less in M2 cells. This enzyme is required for the synthesis of acetyl-CoA in the cytoplasm (Figure 1). Moreover, acetate is used as a precursor for several post-translational modifications, such as histone acetylation, which is associated in macrophages to the transcriptional control under pro-inflammatory conditions.[16,18,19] This is probably the reason why ACS is very active on M1 macrophages. Another question of interest, but not addressed in this work, is related to the potential mechanism of acetate import into the mitochondria. This is a matter of debate, as anions cannot cross the mitochondrial membrane unless they are associated with counter ions that reduce the charge of the molecule. It is supposed that acetate follows the classical way of importing carboxylic acids into the mitochondria. However, due to the kinetics in acetate uptake observed by the authors in M2 macrophages cultured *ex vivo*, it is suggested that the enhanced mitochondrial biogenesis is involved in this increased incorporation of acetate.

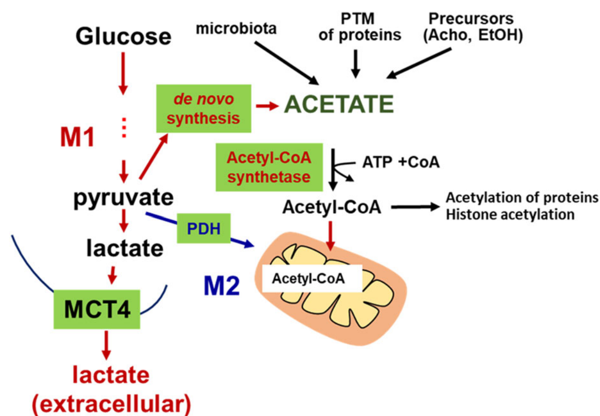

Figure 1. Acetate uptake, biosynthesis and metabolism in macrophages. Acetate can be incorporated in macrophages through specific monocarboxylate transporters. In addition to this, acetate can be produced de novo, from pyruvate. The main enzyme involved in acetate metabolism is Acetyl-CoA synthetase, whose transcription is enhanced in pro-inflammatory M1 macrophages. Intracellular acetate levels are essential for post-translational modifications of proteins, in particular for histone acetylation. PDH: pyruvate dehydrogenase; MCT4: monocarboxylate transporter 4; M1 (red symbols): pro-inflammatory macrophages; M2 (blue symbols): anti-inflammatory/pro-resolution macrophages.

Now, the question is why do atherogenic M2 macrophages incorporate ^{11}C-acetate more significantly than M1? As mentioned above, acetate is widely used by M1 macrophages, probably coming from the *de novo* pathway from pyruvate. These M1 cells are highly glycolytic and have a reduced capacity to use pyruvate *via* the mitochondrial TCA cycle and the oxidative phosphorylation in order to produce ATP (OXPHOS pathway). This is due to the low activity of pyruvate dehydrogenase.[6,20] Since LDH is a very abundant enzyme, the catalyzed reaction will be in chemical equilibrium. This means that both the NAD/NADH and lactate/pyruvate ratios are in equilibrium, forcing lactate to be exported to the extracellular environment *via* the MCT4 transporter (monocarboxylate transporter 4; encoded by the *Slc16a* gene). This protein is also highly expressed in M1 macrophages.[7] Indeed, the use of inhibitors for this transporter in oncology is under clinical trials. Under these conditions, exogenous labeled acetate (for PET/CT or MRI detection) competes with the transport of other monocaboxylates (i.e., lactate and pyruvate that accumulate in the extracellular medium), but also with the flow of pyruvate to acetate, thus reducing the specific activity of the label of acetate. Therefore, M2 macrophages, despite producing and consuming less acetate, are more efficient in incorporating the labeled acetate. However, since acetate can be produced by variable sources (Figure 1), in addition to the pyruvate-dependent pathway, there is the possibility that the intensity of the labeling of atheromatous lesions varies according to the isotopic dilution. Hence, determination of acetate in the serum, interventions on the colonic microbiota to reduce acetate production, and/or the possible combination with additional tracers (i.e., FDG) cannot be disregarded as additional strategies to improve the quality of the ^{11}C-acetate PET/CT imaging of atherogenic lesions.

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