Advanced glycation endproducts and plaque instability: a link beyond diabetes

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This editorial refers to 'Higher levels of advanced glycation endproducts in human carotid atherosclerotic plaques are associated with a rupture-prone phenotype'†, by N.M.J. Hanssen et al., on page 1137

Despite clear advances in the treatment of vascular disease, we are still far from the understanding and valid assessment of the natural history of atherosclerotic plaque. Several studies have shown that the vulnerable plaque is the main culprit for ischaemic cardiac and cerebral events, but reliable indicators of plaque instability remain elusive in clinical practice.[1] This aspect deserves attention since rupture of carotid plaques explains at least 15–20% of all ischaemic strokes or transient ischaemic attacks.[2] Over 15 million people per year suffer strokes worldwide, resulting in 5 million deaths, and an additional 5 million remain permanently disabled.[2] Treatment of carotid artery stenosis by endoarterectomy or stenting can significantly reduce stroke risk, but it is also accompanied by surgery-related morbidity and mortality.[1,2] Moreover, not all carotid artery plaques become symptomatic and result in ischaemic events. In this perspective, the identification of carotid plaques at high risk for neurological events is fundamental to the selection of patients for vascular interventions.

Different processes involved in the progression of atherosclerotic lesions have been associated with plaque vulnerability.[3] Previous seminal investigations have clearly shown that inflammation, proteolysis, and apoptosis are major drivers of plaque complexity and fragility.[3] Pathological features of unstable plaques are represented by an atheromatous thin fibrous cap, large necrotic core, infiltration of inflammatory cells, and scarce calcification. Despite available imaging techniques providing an exhaustive macroscopic characterization of the plaque, the inner mechanisms involved remain to be largely elucidated and characterization of the molecular processes is achieved only at post-operative or post-mortem examination.[1] The assessment of early markers of plaque vulnerability is an attractive challenge to select candidates for mechanism-based therapeutic strategies. Among different biomarkers, advanced glycation endproducts (AGEs) have been linked to the atherosclerotic process and are emerging as a novel signature of atherosclerotic disease.[4] AGEs are a large family of extensively sugar-modified proteins which can be formed in plaques as a consequences of increased metabolic activity.[1,4] It is well established that AGEs are present in atherosclerotic lesions.[5] Of note, inhibition of synthesis of AGEs prevents or attenuates atherosclerosis in experimental models.[4,5] Despite the link between AGEs and atherosclerosis, their precise role in the context of atherosclerotic plaque remains unclear.

Hanssen and colleagues have now shown that AGE accumulation may represent an indicator of plaque vulnerability.[6] The authors found that two major AGEs, the methylglyoxal-derived 5-hydro-5-methylimidazolone (MG-H1) and N^ε (carboxylmethyl)lysine (CML), measured with tandem mass spectrometry, were significantly higher in symptomatic as compared with asymptomatic carotid plaques. MG-H1 and CML were associated with increased levels of the inflammatory cytokines interleukin-8 (IL-8) and monocyte chemoattractant protein 1 (MCP-1), as well as with higher activity of matrix metalloproteinase 9 (MMP-9), an important modulator of plaque collagen content. Immunohistochemistry showed that AGE accumulation occurs in macrophages, is mostly confined to hypoxic regions of the necrotic core, and co-localizes with the apoptotic marker caspase-3 (*Figure 1*). Interestingly, gene expression of glyoxalase-1 (GLO1), the most important methylglyoxal-detoxifying enzyme, was decreased in vulnerable as compared with stable plaques. A provocative finding of the present work is that MG-H1 and CML were independently associated with a vulnerable plaque phenotype, regardless of diabetes. History of diabetes did not affect the link between AGEs and plaque inflammation, and no correlations were found between fasting glycaemia and AGE levels, even after adjustment for glucose-lowering drugs. These findings suggest the possibility that AGEs can also be considered markers of plaque inflammation and instability in non-diabetic individuals. Indeed, the major determinant of intraplaque protein glycation is the high metabolic activity of macrophages, leading to enhanced D-glucose uptake and subsequent formation of AGEs (*Figure 1*). This assumption is supported by the demonstration that positron emission tomography
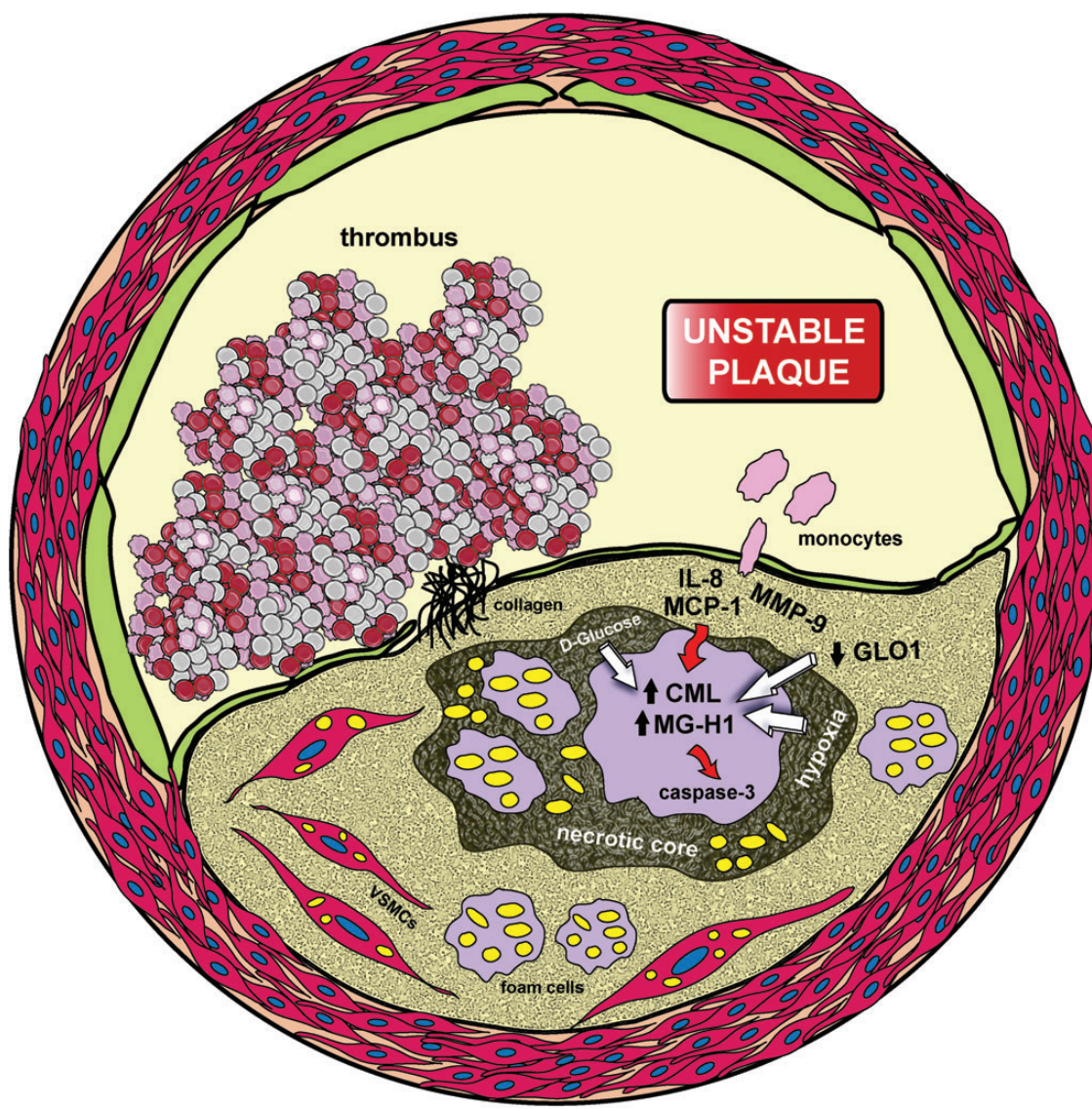

Figure 1 Role of advanced glycation endproducts (AGEs) in plaque vulnerability. The AGEs MG-H1 and CML are synthesized in macrophages of vulnerable carotid plaques due to high glucose uptake. MG-H1 and CML accumulation mostly occurs in the hypoxic region of the necrotic core. Inflammatory cytokines IL-8 and MCP-1 as well as MMP-9 trigger AGE formation and subsequent cell apoptosis via activation of caspase-3. On the other hand, down-regulation of the methylglyoxal-detoxifying enzyme GLO1 contributes to AGE accumulation. CML, N^ϵ (carboxylmethyl)lysine; GLO1, glyoxalase-1; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; MG-H1, 5-hydro-5-methylimidazolone; MMP-9, metalloproteinase-9; VSMCs, vascular smooth muscle cells.

(PET)-based uptake of the glucose analogue [^{18}F]fluorodeoxyglucose (18FDG) is enhanced in symptomatic carotid arteries of non-diabetic patients.[7] Unfortunately, glycated haemoglobin (Hb1$_{Ac}$) was not available in the study by Hanssen *et al.*[6] and it is not possible to rule out that chronic hyperglycaemia might also affect intraplaque glucose content, leading to a further extent of protein glycation.

Another interesting finding of this study is that hypoxia within the necrotic core may trigger AGE formation in macrophages (*Figure 1*). Indeed, MG-H1 and CML co-localized with macrophage-rich and hypoxic regions, as assessed by pimonidazole staining. To investigate the link between intraplaque hypoxia and AGE synthesis further,

MG-H1 and CML levels were measured in human monocytes after exposure to low oxygen concentrations (0.2%). Interestingly, *in vitro* hypoxia enhanced AGE formation mostly via increased methylglyoxal (MGO) levels and GLO1 down-regulation (*Figure 1*). These findings are in line with recent work showing that GLO1-dependent detoxification is an important defender of vascular homeostasis.[8] However, a previous study from the same group showed that polymorphisms in the GLO1 gene are not associated with vascular complications in two Dutch cohorts of patients with normal glucose metabolism, impaired glucose tolerance, and type 2 diabetes mellitus.[9] Therefore, the exact contribution of GLO1 to human

atherosclerosis remains to be elucidated. In this regard, Hanssen *et al.* propose that AGE accumulation may be the effect and not the cause of plaque inflammation.[6] Indeed, the inflammatory *in vitro* effect of tumour necrosis factor-α (TNF-α) on IL-8, MCP-1, or MMP-9 is not dependent on AGEs because the AGE inhibitor aminoguanidine reduced MGO and AGE formation but did not affect IL-8, MCP-1, and MMP-9 expression. Moreover, incubation of monocytes with CML or MGO did not increase the secretion of IL-8, MCP-1, and MMP-9.[6] These findings are in contrast to previous work showing that AGE–RAGE (AGE receptor) signalling leads to an increased oxidative burst via NADPH oxidase and mitogen-activated protein kinases (ERK 1/2) with subsequent activation of nuclear factor-κB (NF-κB) and transcription of inflammatory genes.[10] Thus, the AGE content in the plaque may be the result of a vicious cycle involving inflammation, oxidative stress, AGEs, and NF-κB activation. In contrast, if AGEs are just a consequence of plaque inflammation they cannot be considered early biomarkers of the atherosclerotic process but rather a mirror of the inflammatory milieu. A further interpretation is that the highly antigenic nature of CML primarily triggers an inflammatory response in the subendothelial layer, favouring the atherosclerotic process. This latter hypothesis may not be that unrealistic since autoimmune responses have been postulated as potential drivers of vascular inflammation.[11] In line with this interpretation, the RAGEs were not up-regulated in vulnerable plaques, suggesting that AGE–RAGE interaction may be less relevant than intracellular accumulation of AGEs. However, such a provocative finding of normal RAGE expression in unstable plaques deserves further investigation. A phase II clinical trial demonstrated that pharmacological AGE degradation by the cross-link breaker ALT-711 reduced arterial pulse pressure and improved the compliance of large arteries, two important pre-atherosclerotic features.[12] Another study with benfotiamine prevented both macro- and microvascular endothelial dysfunction and oxidative stress induced by an AGE-rich meal.[13] Moreover, the combined use of benfotiamine and α-lipoic acid normalized the elevated AGEs levels and blocked the enhancement of hexosamine-modified protein formation in monocytes of patients with type 1 diabetes.[14] Based on these studies, AGE formation may represent an upstream event triggering vascular inflammation, oxidative stress, and eventually plaque instability.

In summary, Hanssen and colleagues demonstrate for the first time an intriguing link between AGEs and plaque phenotype. These results imply that AGEs may represent a putative biomarker of rupture-prone plaques, regardless of diabetes. However, several considerations should be taken into account before considering this cardiovascular risk stratification approach. While AGE levels in the plaque strongly correlate with a vulnerable phenotype, it remains unclear whether plasma or skin-derived AGEs similarly reflect this association. This aspect has important clinical implications since the assessment of AGEs in the carotid arteries can be performed only after post-operative examination and it is not applicable as a screening tool. In this context, previous work suggested that measuring AGEs in skin using autofluorescence may provide important information on risk stratification in diabetic patients. This study involving 972 diabetic patients showed that the addition of skin AGEs to the UKPDS risk engine resulted in re-classification of 27% of the patients from the low- to the high-risk group.[15] The 10-year cardiovascular event rate was higher in patients with a UKPDS score >10% when skin AGEs were above the median (56% vs. 39%).

Undoubtedly, this work has shed some light on the intriguing issue of AGE levels as early biomarker of plaque instability. However, additional pre-clinical studies are mandatory to understand whether AGE inhibition may prevent or delay atherosclerotic complications.

Conflict of interest: none declared.

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