Measurement of 5-hydroxy-2-aminovaleric acid as a specific marker of metal catalysed oxidation of proline and arginine residues of low density lipoprotein apolipoprotein B-100 in human atherosclerotic lesions

J Pietzsch, R Bergmann

Oxidative modification of low density lipoprotein (LDL) apolipoprotein B-100 (apoB-100) is regarded as a crucial event in atherogenesis. Direct oxidation of apoB-100 amino acid residues is thought to result in the formation of new epitopes that are specifically recognised by scavenger receptors. However, the nature of such new epitopes is still a matter of debate. One potential pathway for direct oxidation of apoB-100 involves megaloxiperoxidase, which finally generates specific oxidation products, such as 3-chlorotyrosine and 3-nitrotyrosine. All of these compounds have been found in LDL apoB-100 recovered from human atherosclerotic lesions. These findings support the hypothesis that pathways involving metal catalysed oxidation of LDL apoB-100 are of pathological importance in atherogenesis.

γ-Glutamyl-semialdehyde (γGSA) is a major product of the metal catalysed oxidation of apolipoprotein B-100 (apoB-100) proline and arginine residues. On reduction, γGSA forms 5-hydroxy-2-aminovaleric acid (HAVA). This report describes the application of HAVA measurement to characterise the formation of γGSA in low density lipoprotein (LDL) recovered from human atherosclerotic lesions. HAVA concentrations were greatly increased in LDL from early (mean, 10.25; SD, 3.49 mol/mol apoB-100; p < 0.01), intermediate (mean, 11.18; SD, 2.37 mol/mol apoB-100; p < 0.01), and advanced (mean, 9.91; SD, 2.15 mol/mol apoB-100; p < 0.01) lesions, when compared with LDL from normal aortic tissue (mean, 0.05; SD, 0.01 mol/mol apoB-100). These findings support the hypothesis that pathways involving metal catalysed oxidation of LDL apoB-100 are of pathological importance in atherogenesis.

METHODS AND MATERIALS

Lesional LDL was isolated from the intima of normal and atherosclerotic specimens of human thoracic aortas obtained at necropsy within 10 hours of sudden death (20 male accident victims, aged 26 to 45 years; no signs of severe acute diseases). Normal and atherosclerotic tissue (fatty streaks, intermediate, and advanced plaques) was classified according to the criteria of the pathobiological determinants of atherosclerosis in youth study. Samples were processed in subdued light to prevent photooxidation. Aortic intima was dissected from the medium and homogenised for two hours at 4°C (30 mg wet tissue/ml phosphate buffered saline, pH 7.2). All buffers and solutions were degassed and stored under argon. Furthermore, all buffers and solutions were supplemented with 100μM diethylenetriaminepentaacetic acid, 100μM butylated hydroxyanisole, and 100μM 3-amino-1,2,4-triazole. Lesional LDL (density, 1.006–1.063 g/ml) was then isolated by very fast ultracentrifugation at 120 000 revolutions/minute, corresponding to 625 000 × g, with a run time of 100 minutes at 18°C. Delipidation of LDL, formation of HAVA by reduction of γGSA with sodium borohydride, and enzymatic hydrolysis of apoB-100 were described previously. The free amino acids from hydrolysates were derivatised to their N-ethoxycarbonyl ethyl ester derivatives, and analysed by GC-MS, as described elsewhere. The HAVA content in all samples is expressed as mol/mol apoB-100 (intra-assay coefficient of variation (CV), < 4.5%; interassay CV, < 6.1%).

RESULTS AND DISCUSSION

Figure 1 shows the HAVA content of intima from various stages of lesion evolution as determined by sensitive and specific GC-MS analysis. The HAVA content was significantly higher in LDL recovered from all types of lesions when compared with normal aortic tissue (paired Student’s t test). The overall yield of HAVA that has been found in lesional LDL (mean HAVA content, 10.45; SD, 0.66 mol/mol apoB-100; 328/10 000 proline plus arginine residues) is remarkably high, and indicates that proline and arginine residues are good targets for oxidative modification.
for metal catalysed oxidative attack in atherosclerotic tissue. Control experiments indicated that sample processing was unlikely to have contributed to HAV A formation as an artifact (data not shown in detail). The HAV A content of both atherosclerotic and normal aortic tissue exceeded the physiological concentrations of HAV A formerly found in native plasma LDL obtained from healthy normolipidaemic subjects (mean, 0.012; SD, 0.004 mol/mol apoB-100; 0.4/10 000 proline plus arginine residues). Furthermore, the findings are consistent with former data showing increased HAV A content (mean, 0.063; SD, 0.02 mol/mol apoB-100; 2.0/10 000 proline plus arginine residues) in circulating LDL recovered from hypercholesterolaemic subjects who were at high atherosclerotic risk. The experiments support the hypothesis that a pathway involving metal catalysed oxidation of LDL apoB-100 may be of pathological importance in atherogenesis. However, additional work is needed to understand the specific consequences of γGSA formation for the metabolic fate of apoB containing lipoproteins in vivo.

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REFERENCES

Take home messages
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- These findings support the hypothesis that pathways involving metal catalysed oxidation of LDL apolipoprotein B-100 are of pathological relevance for atherogenesis.

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