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Effect of vitamin e and tempol on low density lipoprotein oxidation at lysosomal pH

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Background

a-Tocopherol (vitamin E) is well known to inhibit the oxidation of low density lipoprotein (LDL), but did not protect against cardiovascular disease in the large clinical trials. We have shown (Wen & Leake, 2007) that LDL is oxidised in the lysosomes of macrophages.

Purpose

The aim of this study to investigate of additional Tempol to LDL enriched with a-tocopherol can inhibit oxidised LDL which induced by different oxidative stress at lysosomal pH. We determined the effect of a-tocopherol on LDL oxidation at lysosomal pH and also the effect of the antioxidant Tempol, a stable nitroxyl radical.

Method

Blood was obtained from healthy volunteers and plasma incubated at 37 °C with 1% (v/v) of dimethylsulphoxide (DMSO) containing 100 mM (+) a-tocopherol for 3 hours (final concentration 1 mM) or with 1%DMSO alone as a control. LDL was then isolated by sequential ultracentrifugation (Esterbauer et al., 1991).

LDL (50µg protein/ml) was incubated with FeSO₄ or CuSO₄ in a sodium acetate buffer pH 4.5 in automatic spectrophotometers and conjugated dienes (oxidised fatty acids) measured at 234 nm. Cu⁺ was measured using bathocuproinedisulfonic acid and Fe²⁺ bathophenanthrolinedisulfonic acid.

Result

LDL enriched in a-tocopherol was oxidised more slowly than control LDL by Cu²⁺ at pH 7.4, as expected, but was oxidised faster by Cu²⁺ or Fe³⁺ at pH 4.5. a-Tocopherol-enriched LDL increased the rate of reduction of Cu²⁺ to Cu⁺ and Fe³⁺ to Fe²⁺. This would have formed the a-tocopheroxyl radical, which can initiate LDL oxidation by converting polyunsaturated lipids into lipid radicals, and metal species which are more active in breaking down lipid hydroperoxides. Tempol inhibited all phases of LDL oxidation by Cu²⁺ at pH 7.4, but only inhibited effectively the later phases of oxidation of both control and a-tocopherol-enriched LDL at pH 4.5. Tempol might inhibit effectively the oxidation of the phospholipid monolayer, but not the cholesteryl ester core, of LDL at pH 4.5.

Conclusion

These findings might help to explain why the large clinical trials of a-tocopherol did not protect against
cardiovascular disease.