Effects of in vitro addition of captopril on copper-induced low density lipoprotein oxidation

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Low density lipoprotein (LDL) was incubated with 20 μM of the angiotensin converting enzyme (ACE) inhibitors captopril, fosinopril and quinapril or ethanol. Oxidation of LDL was initiated by addition of CuSO₄ and monitored for production of conjugated dienes, thiobarbituric acid reactive substances (TBARS) and lipid peroxides. The inhibition of production of conjugated dienes was expressed as the lag phase in minutes. The lag phase for control samples was 55.2 ± 6.1 (mean ± s.e. mean) min and for captopril 86.4 ± 7.0 min (P = 0.0058). Quinapril had a small but non-significant effect, fosinopril and ethanol had no effect. LDL incubated with captopril showed only 13.8% of TBARS and 22.7% of lipid peroxides produced by control (100%) after 1 h. Increasing concentrations of captopril showed a linear increase in the lag phase. We conclude that captopril increases the resistance of LDL to copper-induced oxidation.

**Keywords** low density lipoprotein oxidation ACE inhibitors captopril quinapril fosinopril

**Introduction**

Oxidatively modified low density lipoproteins (ox-LDL) have been suggested to be important in the pathogenesis of atherosclerosis. Ox-LDL are taken up rapidly via the scavenger receptor expressed by macrophages. This uptake is said to explain the accumulation of lipids in the fatty streak, which is thought to precede more advanced atherosclerotic lesions [1]. A possible way of inhibiting or delaying this oxidation would be to administer antioxidants. Recently it was found that two inhibitors of angiotensin converting enzyme (ACE), captopril and fosinopril, both reduce experimentally induced atherosclerotic lesions in hypercholesterolaemic mini-pigs [2], although the effect was much more pronounced for captopril. This effect was supposed to be due partly to free radical scavenging by the sulphhydryl (SH-) group in the captopril molecule acting as an antioxidant.

The aim of this study was to test the hypothesis that LDL incubated in vitro with captopril or other ACE inhibitors, could delay the oxidation of LDL.

**Methods**

Fresh whole blood was obtained from seven healthy volunteers by venepuncture after an overnight fast. The blood was collected in EDTA (1.4 mg ml⁻¹) containing tubes and immediately cooled. Plasma was centrifuged at 1700 g for 15 min. LDL was obtained by preparative ultracentrifugation within a density gradient of 1.020–1.050 g cm⁻³ [3]. EDTA was present throughout the isolation process. Before the oxidation experiments the LDL was gel-filtered (Sephadex G-25 M, Pharmacia LKB, Sweden) twice, or dialysed against 100 volumes of 0.01 M phosphate buffer (NaCl 0.16 M, pH 7.4) for 22 h.

The dialyzed LDL, 50 μg ml⁻¹, was incubated with 20 μM of the ACE inhibitors captopril, fosinopril, or quinapril at room temperature. Quinapril was dissolved in 95% ethanol and captopril and fosinopril were dissolved in phosphate buffer solutions. LDL incubated with phosphate buffer or ethanol served as controls. CuSO₄ 10 μM (final concentration) was added to initiate oxidation and the change in absorb-
ance was monitored at 234 nm every 5 min for 2–4 h at +37°C [4]. The lag phase (min) was defined as the intercept of the slope during the propagation phase with the initial absorbance axis. The intra-assay error was less than 6%.

Gel-filtered LDL 50 μg ml⁻¹ was then incubated with increasing concentrations of captopril. Oxidation was initiated and monitored as described above.

Determination of thiobarbituric acid reactive substances (TBARS) was performed as described by Buege & Aust [5] and lipid peroxides according to El-Saadani [6]. In summary, dialyzed LDL 100 μg ml⁻¹ was incubated with 40 μM captopril, fosinopril, quinapril or ethanol for 30 min at room temperature. CuSO₄ 10 μM was then added to initiate oxidation. Aliquots were drawn at time 0, 1, 2, 3, 6 and 24 h. TBARS are expressed as TBARS nmol mg⁻¹ LDL protein using an extinction coefficient of 1.56 × 10⁻⁵ M⁻¹ cm⁻¹ and lipid peroxides as nmol mg⁻¹ LDL protein with an extinction coefficient of 2.46 × 10⁻⁵ M⁻¹ cm⁻¹. Total LDL protein was determined according to Lowry [7].

Values are given as means and standard error of the mean (s.e. mean). Statistically significant differences between control and the ACE inhibitors were considered at P < 0.05 using Student’s unpaired t-test.

Results

When LDL was incubated with fosinopril or ethanol in vitro, no prolongation of the lag phase was seen as compared with control (Table 1). A slight, although not significant, increase was observed with quinapril. Captopril induced a prolongation of the lag phase by 31.2 ± 3.3 min (P = 0.0058). The increase in TBARS and lipid peroxides during the first hour was significantly less in LDL incubated with captopril as compared with control (Table 1). We found no differences in TBARS or lipid peroxide generation between active agents and control during the later stages of oxidation.

A pool of LDL was then incubated with different concentrations of captopril and tested for the effects on lag phase (Figure 1). The mean lag phase for control LDL was 42.9 ± 1.9 min. With increasing concentrations of captopril, the increase in lag phase was approximately linear. The lag phase of LDL incubated with 15 μM captopril was 75.6 ± 0.5 min and captopril at a concentration of 50 μM prolonged the lag phase by 275 ± 5.5 min compared with control LDL.

Discussion

The mechanisms responsible for the dramatic antioxidant effect of captopril with a pronounced pro-

![Figure 1](image_url)  
**Figure 1** Effects of different concentrations of captopril on the lag phase. LDL 50 μg ml⁻¹ was incubated with increasing concentrations of captopril (5–50 μM). Oxidation was initiated with 10 μM CuSO₄ and the absorbance at 234 nm was followed spectrophotometrically. Bars indicate mean and s.e. mean of three experiments.

| Table 1 Effects (mean ± s.e. mean) of ACE inhibitors or ethanol on LDL oxidation |
|---------------------------------|-----------------|--------------|
| **Lag phase (min)**            | **Lipid peroxides (nmol mg⁻¹)** | **TBARS (nmol mg⁻¹)** |
|                                 | 1 h | 2 h | 3 h | 1 h | 2 h | 3 h |
| Control                         | 55.2 ± 6.1 | 119 ± 24 | 265 ± 26 | 250 ± 10 | 27.6 ± 3.4 | 54.1 ± 1.8 | 55.3 ± 5.7 |
| Captopril                       | 86.4 ± 5.0**| 27 ± 6** | 200 ± 14 | 249 ± 24 | 3.8 ± 2.0** | 47.4 ± 2.8 | 52.6 ± 4.5 |
| Fosinopril                      | 55.6 ± 5.9 | 106 ± 18 | 222 ± 5 | 235 ± 8 | 26.6 ± 6.0 | 53.9 ± 3.0 | 52.0 ± 4.3 |
| Quinapril                       | 66.7 ± 7.4 | 86 ± 33 | 263 ± 7 | 270 ± 11 | 23.6 ± 4.5 | 53.9 ± 3.0 | 53.0 ± 4.3 |
| Ethanol                         | 55.4 ± 7.4 | 100 ± 22 | 258 ± 6 | 256 ± 10 | 29.4 ± 5.9 | 59.1 ± 2.9 | 58.9 ± 5.5 |

*P < 0.05, **P < 0.01, ***P < 0.001 vs control. For lag phase determination (n = 7), 50 μg ml⁻¹ LDL was incubated with 20 μM of the ACE-inhibitor or ethanol. Oxidation was initiated with 10 μM CuSO₄. For TBARS (n = 7) and lipid peroxides (n = 6) twice the concentration of all components were used.
longation of the lag phase are not completely understood. Extensive studies on the antioxidant action of captopril have been carried out by Chopra et al. [8]. According to them the thiol-group is the site of free radical scavenging. It has also been discussed that captopril itself might possess copper-chelating properties [9]. Our experiment supports the findings of Chopra et al. [8]. The clinical relevance of our observations is difficult to evaluate at present. Based on our earlier detailed pharmacokinetic studies of captopril and its effects on blood pressure in hypertensive patients [10], the incubation concentrations used in the present study (5–50 μM) may be somewhat high. We measured plasma concentrations in these patients up to 5 μM (2–3 h after drug administration) but the captopril doses used (25–75 mg day⁻¹) were lower than those now recommended (150 mg day⁻¹) for the prevention of progression of left ventricular dysfunction [11]. Thus, the incubation concentrations used here may be relevant both physiologically and clinically. Our earlier experience with captopril (as compared with fosinopril) as an anti-atherogenic agent in cholesterol-fed pigs [2] may also indicate that the SH-group in the captopril molecule is responsible for the anti-atherosclerotic effect.

Further investigations, including in vivo studies, are necessary to confirm the clinical relevance of a prolonged lag phase. This may be relevant to the future role of captopril as an antioxidant agent and its potential as an anti-atherogenic agent.

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References


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