AN INVESTIGATION OF THE EFFECTS OF CELLULAR PROOXIDANTS ON CHOLESTEROL OXIDATION IN A LIPOSOMAL MODEL SYSTEM

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BACKGROUND

In recent years, increasing interest has been expressed in the occurrence of cholesterol oxidation products (COPs) in animal-derived foods. Evidence suggests that COPs may be involved in atherosclerosis, and, therefore, their occurrence in the food chain is of some concern. It has been shown that cholesterol oxidation is accelerated by factors which promote the oxidation of unsaturated fatty acids, such as heating, exposure to light, prolonged storage and packaging (Paniangvait *et al.*, 1995). In meat, intrinsic factors such as free metal ions and haem proteins (myoglobin, haemoglobin) also accelerate the lipid oxidation process (Kanner, 1994). Studies have shown that iron, copper and myoglobin are capable of catalysing cholesterol oxidation *in vitro* (Terao *et al.*, 1985); Galaris *et al.*, 1988; Huber *et al.*, 1995), and may be expected to contribute to cholesterol oxidation in meats. However, little information is available concerning the effects of cellular prooxidants on cholesterol oxidation in meat or muscle systems.

OBJECTIVES

The aim of this study was to investigate the effect of various concentrations of prooxidants (iron/ascorbate, copper, metmyoglobin/H_2O_2), and to compare their effectiveness, on cholesterol oxidation in a liposomal model system.

METHODS

Liposomes were prepared according to the method of Maerker & Jones (1991). Multilamellar vesicles (MLVs) were prepared using dipalmitoylphosphatidylcholine (70.5mg), dipalmitoylphosphatidylglycerol (23.1mg), chicken muscle phospholipids (10mg) and cholesterol (24.8mg) in a final volume of 16ml. Chicken muscle phospholipids were extracted by the method of Marmer & Maxwell, (1981). The phospholipids and cholesterol were dissolved in chloroform (3ml). The solution was vortexed, and dried under nitrogen. Dried lipids were hydrated in water (8ml) and liposomes were allowed to swell for 3h at 55°C. Liposomes were filtered through a 0.4µm pore polycarbonate membrane and chromatographed on a Sephadex G50-80 column to ensure uniform vesicle size. The phospholipid concentration of the final liposomal solution was checked by determination of the phosphorous content (Anderson & Davis, 1982).

Cholesterol oxidation in control liposomes was initiated by the addition of 5mM 2,2'-azobis (2-amidinopropan) dihydrochloride (AAPH). Aliquots of liposomes were incubated at 37°C in a shaking water bath. Experimental groups were incubated with AAPH and $FeSO_4$/ascorbic acid, $CuCl_2$ or metmyglobin/H_2O_2 at various concentrations. The cholesterol and 7-ketocholesterol content were determined prior to incubation. 7-ketocholesterol was determined at intervals during the incubation.

Cholesterol and 7-ketocholesterol were extracted from liposomes as described by Maerker & Jones (1991), and analysed by a GC method as described previously (Galvin *et al.*, 1998).

All data were subjected to analysis of variance (ANOVA). Where significant differences were detected, means were compared by the method of least significant difference (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

The effects of increasing concentrations of prooxidants in the model system on cholesterol oxidation are shown in Figure 1. Iron/ascorbate concentrations of 15 and 30µm did not increase 7-ketocholesterol (7-keto) levels compared to control values up to an incubation time of 12h. After this point, 7-keto levels were significantly ($P<0.05$) higher than control values, at both concentrations. The addition of $4x10^{-4}$M iron/ascorbate significantly ($P<0.05$) increased 7-keto compared to control values at all time points. In general, no significant differences were observed between the 15 and 30µM concentrations. At all time points, 7-keto levels were significantly higher ($P<0.05$) at the $4x10^{-4}$M concentration compared to other concentrations. The addition of copper did not increase 7-keto levels at the 15 and 30µm concentrations, but the highest copper concentration (2.5mM) significantly ($P<0.05$) increased levels over control values, following incubation for 6h. Metmyoglobin/H_2O_2 had no effect on cholesterol oxidation up to 12h of incubation, at all concentrations used. All concentrations significantly ($P<0.05$) increased 7-keto levels compared to the control after this point. In general no significant differences were observed between different concentrations of metmyoglobin/H_2O_2.

The comparative effects of equimolar concentrations (30µM) of iron/ascorbate, copper and metmyoglobin/H_2O_2 are shown in Figure 2. Overall, iron/ascorbate resulted in significantly ($P<0.05$) higher levels of 7-keto compared to copper. Levels were either similar to or significantly ($P<0.05$) higher than levels in the metmyoglobin/H_2O_2 group. Overall, iron/ascorbate showed the greatest prooxidant activity, followed by metmyoglobin. Copper had a very weak prooxidant effect, and was only effective at high very concentrations.

CONCLUSIONS

Iron/ascorbate, copper and metmyoglobin/H_2O_2 accelerated cholesterol oxidation in a liposomal model system. At equimolar concentrations, iron/ascorbate showed that greatest prooxidant effect, followed by metmyoglobin, while copper showed a very weak prooxidant effect. The results suggest that transition metals and haem proteins could contribute to the oxidative degradation of cholesterol in meats.

ACKNOWLEDGEMENT

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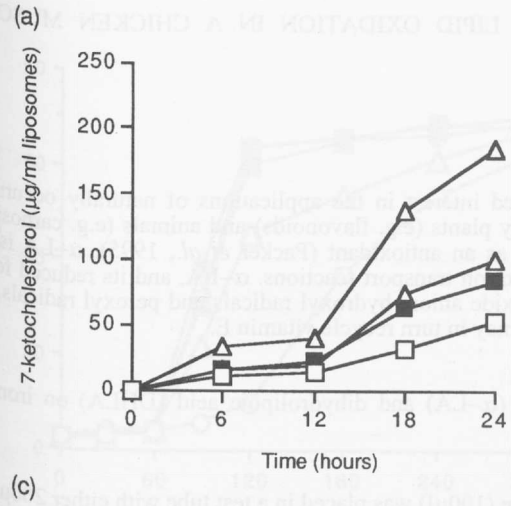

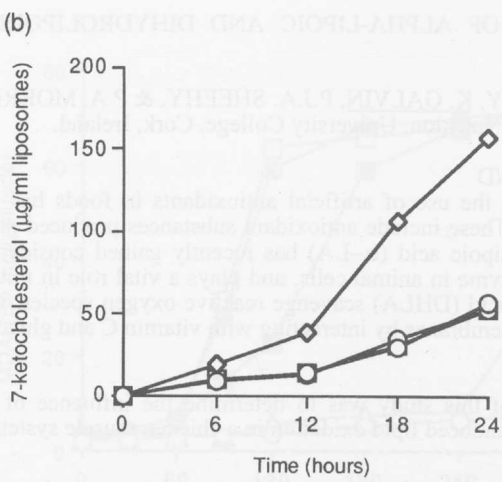

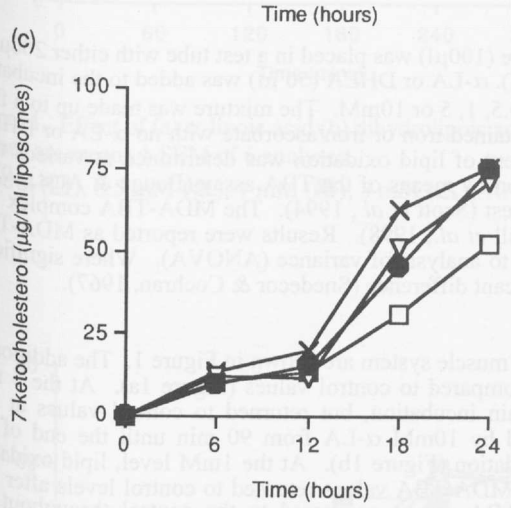

Figure 1. Effect of (a) iron-ascorbate, (b) copper and (c) metmyglobin/H_2O_2 on the formation of 7-ketocholesterol in liposomes exposed to AAPH (5mM), during incubation at 37°C.
Values are means of 3 analysis performed in duplicate.
Control (AAPH only) (□)
15µM (■); 30µM (△); 400µM (▲) iron/ascorbate
15µM (○); 30µM (●); 2.5mM (◇) copper
15µM (◆); 30µM (▽); 45µM (X) metmyoglobin/H_2O_2.

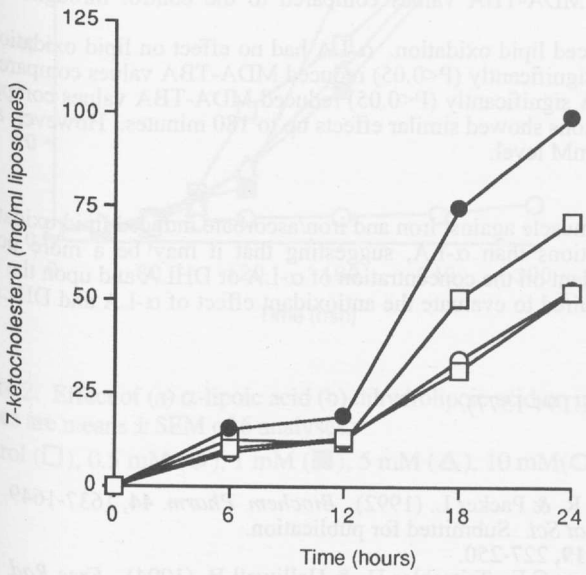

Figure 2. Comparison of the effects of equimolar concentrations (30µM) of iron/ascorbate (●), copper (○) and metmyglobin/H_2O_2 (■) on the formation of 7-ketocholesterol in liposomes exposed to AAPH, during incubation at 37°C.
Values are means of 3 analyses performed in duplicate.
Control (□) = AAPH only.