the groups studied. Therefore copper may be involved in the deficiency called Menkes disease, gross arterial disease is a constant [3, 4]. In the human genetic disease of copper work has shown that atherosclerosis is associated with low liver and heart copper. Zinc levels remained relatively atherosclerosis, IHD

Studies in copper status and atherosclerosis

G. D. KINSMAN,* A. N. HOWARD,* D. L. STONE† and P. A. MULLINS‡

*Department of Morbid Anatomy, University of Cambridge, Addenbrookes Hospital, Cambridge CB2 2QQ, U.K., and †Department of Cardiology, Papworth Hospital, Papworth Everard, Cambridge CB3 8RE, U.K.

Summary

Findings of this paper indicate that leucocyte copper has a significant link with the level of atherosclerosis found within the groups studied. Therefore copper may be involved in the mechanisms associated with ischaemic heart disease (IHD).

Introduction

Copper deficiency has been implicated in the aetiology of atherosclerosis. IHD [1] and aortic aneurysms [2]. Previous work has shown that atherosclerosis is associated with low liver and heart copper. Zinc levels remained relatively constant [3, 4]. In the human genetic disease of copper deficiency called Menkes disease, gross arterial disease is a main feature [5]. However, the papers mentioned are limited to post mortem studies. To study copper status on the progression of the disease a minimal invasive technique on living subjects is required. None of the current methods used to assess body copper status are ideal [6]. The majority of copper in plasma and serum is in the form of ceruloplasmin. Ceruloplasmin is subject to interindividual variation [7] and is an acute phase reaction protein. So, levels rise in disease and pregnancy which are totally unrelated to body copper levels. Additional

Abbreviations used: IHD, ischaemic heart disease; CAD, coronary artery disease; GFAAS, graphite furnace atomic absorption spectrophotometry.

Overall, it is obviously difficult to draw any but the most speculative conclusions. These results do encourage further investigation in several directions. Firstly, it may be possible to reduce some responses to oxidants by augmenting intracellular glutathione concentration. Secondly, manipulation of glutathione may also affect responses to other agonists, such as peptides. It will be of particular interest to determine whether some aspect of the effect of these peptides may be linked to the activation of intracellular oxidative processes [7].

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BIOCHEMICAL SOCIETY TRANSACTIONS
Weighting for each artery; for the i th lesion = 1 - (g/4)

Left Coronary Index (LCI)  
= (\[W_i(W_i + W_i + W_i + W_i)] + W_i + W_i + W_i) / 5

Right Coronary Index (RCI) = (\[W_i(W_i + W_i + W_i + W_i)] / 3

Total Coronary Index = (5 × LCI + 3 × RCI) / 8

The scoring ensured that lesions were graded according to position and stability, with parallel lesions adding in the index and serial lesions multiplying. The index was adjusted slightly depending on the number of 'end' vessels. The data were arranged in two groups: samples (n = 8) with advanced CAD (angiogram score < 0.4), and controls (n = 8) with relatively normal arteries (angiogram score > 0.6).

Results

Significant statistical differences were deduced between leucocyte copper compared with angiogram score correlation (r = 0.67, 0.01 > P > 0.001) (Fig. 1); pooled controls and samples (0.60 ± 0.17 compared with 0.28 ± 0.12 μg Cu/l per 10^6 cells, P = 0.005%) (Fig. 2); and age paired (± 5 yrs) controls and samples (0.331 ± 0.17 μg Cu/l per 10^6 cells, P = 0.14%) (Fig. 3).

Discussion

The limited amount of data was mainly due to the restrictions placed on the population to be studied (see Protocol).

![Fig. 1. Leucocyte copper versus angiogram score in male non-smokers](image1)

Sample size, n = 18; correlation, r = 0.67 (0.01 > P > 0.001).

![Fig. 2. Male non-smokers copper level](image2)

Far angiogram scores < 0.4 and > 0.6; pooled t-test:

P = 0.005%, ΔCu > 0.06 A.S., mean = 0.60 ± 0.17; ΔCu < 0.4 A.S., mean = 0.25 ± 0.8.

While stating copper levels as 'μg Cu/l per 10^6 cells' care was taken to monitor that clumping of cells did not occur so reducing the possibility of bogus copper levels. Control mean copper value of 0.60 ± 0.17 μg Cu/l per 10^6 cells compares well with other literature values [9, 11] namely, 0.79 ± 0.43 and 0.73 ± 0.24.

Note that there is no data showing 'low' leucocyte copper having a 'low' incidence of CAD or 'high' level of copper being a result of a 'high' incidence of CAD. The highly significant differences of copper concentrations shown between the control group and the CAD group indicate that leucocyte copper has a significant link with the level of atherosclerosis found. Prolonged suboptimal levels of copper could impair Cu-enzyme-dependent functions [12].

The copper-dependent enzyme lysyl oxidase is probably the most important enzyme with respect to aortic integrity since its major role is in the synthesis of cross linking of elastin and collagen. Deficiency could result in vascular rupture [13, 14]. More specific to leucocytes, however, is superoxide dismutase which performs a protective anti-oxidant function and so protects cell membranes from free radical damage [15, 16]. In addition to this is the fact that lipid peroxides are important in the progression of atherosclerosis [17]. A great deal of importance has been given to lipids in atherogenesis. However, serious doubts about the validity of the lipid hypothesis have recently been published [18, 19]. Findings of this study imply that copper may be involved in the mechanisms associated with IHD.

Effects of human low-density lipoproteins on human polymorphonuclear leucocytes in vitro

C. BONNEAU, R. COUDERC, M. ROCH-ARVEILLER, J. P. GIROUD and D. RAICHVARG

Laboratoire de Biochimie A et Département de Pharmacologie Clinique, CNRS URA 395, Hôpital Cochin, 27 rue du Faubourg Saint Jacques, F-75674 Paris Cedex 14, France

Introduction

The pathogenesis of atherosclerosis is complex and multifactorial. Polymorphonuclear leucocytes (PMN) and monocytes have been observed in the earliest lesions of atherosclerosis in animal models and in man [1]. During the respiratory burst, PMN generate highly reactive oxygen-derived free radicals that may cause tissue damage and contribute to the development of intimal lesions. Low-density lipoproteins (LDL) have been described as activators of monocyte oxidative metabolism and lysosomal enzyme release [2, 3] and it has been reported that PMN from hyperlipidemic athero-prone subjects show increased oxidative metabolism [4]. The aim of our study was thus to evaluate the effect of LDL on human PMN oxidative metabolism and migration in vitro.

Materials and methods

LDL (1.030 < d < 1.050 g/ml) were isolated from pooled serum from fasting subjects by ultracentrifugation at 100 000 g for 18 h at 4°C [5]. They were then dialysed exhaustively against 0.015 M phosphate buffer, sterilized by filtration and stored under nitrogen at 4°C. LDL concentration was expressed in mmol of cholesterol/l. PMN were isolated from the heparinized venous blood of healthy subjects, using a two-step procedure with Ficoll-Hypaque and polyvinylalcohol [6].

Superoxide (O$_2^-$) production was measured in terms of ferricytochrome c reduction [7]. The results were expressed in nmol of O$_2^-$ released/10$^6$ cells. The stimulating agents used were phorbol 12-myristate 13-acetate (PMA) at a final concentration of 10$^{-7}$ mol/l and opsonized zymosan (OZ) at a final concentration of 1 mg/ml. PMN random migration and chemotaxis towards formyl methionyl leucyl phenylalanine (fMLP, 10$^{-7}$ mol/l) and C5a complement fraction (in 4-fold-diluted normal serum) were assessed using the agarose technique [8]. The results were expressed in arbitrary units.

Results

Effect of LDL on PMN superoxide generation. When PMN were preincubated for 5 min with LDL, O$_2^-$ generation over the following 15 min was enhanced in a dose-dependent manner (Fig. 1). O$_2^-$ generation was moderately stimulated at low LDL-cholesterol concentrations but reached more than 50% of values for PMA-stimulated PMN at an LDL concentration of 7.5 mmol cholesterol/l.

The kinetics of LDL-stimulated O$_2^-$ production showed that PMN were no longer stimulated after 15 min of contact with LDL.

Superoxide generation in response to PMA (10$^{-7}$ mol/l) was dramatically decreased in PMN preparations pre-incubated for 5 min with LDL. The inhibitory capacity of LDL was dose-dependent (Fig. 1) and was statistically significant at low cholesterol concentrations. However, when the incubation of PMN in the presence of both LDL and PMA was prolonged to 30 min, O$_2^-$ generation showed a late increase. OZ-stimulated O$_2^-$ generation by PMN was not modified by LDL, regardless of the preincubation or incubation times.

Effect of LDL on PMN migration. LDL modified spontaneous PMN migration only at high concentrations. The chemotactic response of PMN to fMLP or C5a was also inhibited by LDL in a dose-dependent manner. The effect was particularly marked when C5a was used as the chemotactic factor (Fig. 2).

Discussion

Previous studies have shown that oxidized lipoproteins are cytotoxic and alter cell functions in vitro [9]. However, we...