The role of thiols in oxidation of low-density lipoprotein by macrophages

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The oxidation of low-density lipoprotein (LDL) by macrophages, smooth muscle cells and endothelial cells, within the vessel wall, may play a central role in the development of atherosclerotic lesions [1]. Proposed mechanisms of cell-mediated oxidation, include attack by lipoygenases [2] or generation of superoxide [3] but these mechanisms have proved controversial [4,5]. Nitric oxide protects against cell-mediated oxidation of LDL [6], but may, in the presence of superoxide, oxidation of LDL was performed as described [11]. The production of extracellular thiols, in the presence of transition metals, appears to be one mechanism by which macrophages [9] and smooth muscle cells [10] may oxidize LDL.

We have shown that the production of thiols, and the oxidation of LDL by human (THP-1) macrophages, is dependent upon the presence of cystine in Hams F10 culture medium. Macrophage-mediated oxidation of LDL was performed as described [11], and measured as TBARS (nmol/mg LDL) and Relative Electrophoretic Mobility. Production of extracellular thiols, measured as described [9,11], was essentially linear during a 24h incubation. Increasing concentrations of cystine, added to Hams F10 formulated without cystine, increased both thiol production and LDL oxidation (without affecting cell-free oxidation of LDL). Oxidation of LDL appeared to require a threshold level of thiol production, before plateauing at higher concentrations of cystine. Added to Hams F10 culture medium, Macrophage-mediated oxidation of LDL by macrophages, THP-1 macrophages, therefore appear relatively unaffected by changes within the glutathione pool of THP-1 macrophages.

Table 1

| Effect of 3-DZA on extracellular thiol levels and LDL oxidation by THP-1 macrophages |
|-----------------------------------|----------------|------------------|
| Thiol (µM) | Macrophages | Cell-free |
| No addition | 41.3±0.6 | 3.2±0.14 | 1.5±0.06 |
| DZA | 39.9±1.7 | 3.25±0.14 | 1.61±0.07 |
| DZA+BSO | 47.3±3.4 | 3.38±0.06 | 1.63±0.06 |
| DZA+BCNU | 41.8±0.8 | 3.33±0.09 | 1.64±0.05 |
| U | 51.3±0.9 | 3.23±0.10 | 1.65±0.05 |

Incubation of THP-1 macrophages in Hams F10, without cystine, depletes the intracellular glutathione content [11]. We therefore examined the effect of agents which modulate intracellular glutathione concentrations upon thiol production and LDL oxidation. Buthionine sulfoximine (BSO), inhibited glutathione synthesis, but did not significantly affect either LDL oxidation or thiol production [11]. BCNU (1,3-bis(2-chloroethyl)-1-nitrosurea), an inhibitor of glutathione reductase [12], reduced thiol production by a small but significant amount (12% at 1µM, n=3, p<0.05) but did not affect oxidation of LDL, presumably because the third production did not decrease below the threshold level required for oxidation. This effect was not enhanced by the addition of higher concentrations of BCNU, or by the presence of BSO. Addition of 3-deazaadenosine (DZA), an inhibitor of the S-adenosylmethionine pathway [13], did not decrease thiol production or LDL oxidation, even in combination with BSO and/or BCNU (Table 1).

Production of extracellular thiol, and of LDL oxidation, therefore appear relatively unaffected by changes within the glutathione pool of THP-1 macrophages.