110 Superoxide Dismutase Activity of Antibodies Purified from the Human Arteries and Atherosclerotic Lesions


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The cumulative damage associated with exposure to oxidant stress is thought to contribute to ageing and diseases such as atherosclerosis [1]. Oxidative damage is mediated by active oxygen species which are generated as a side-product of metabolism. The superoxide anion (O_2^-) is a reducing agent and although relatively harmless in itself it can undergo spontaneous dismutation to reactive oxygen species such as OH^-. Protection against tissue damage by OH^- is afforded by antioxidant enzyme systems which include the superoxide dismutase (SOD) family of enzymes [see reference 2 for a review]. Tissue-specific isozymes of SOD are expressed both extracellularly (in the plasma) and intracellularly in most aerobic organisms and their activity is generally high in tissues where oxygen radicals are present at elevated levels, e.g., lung, liver, brain and plasma. Studies of mammalian species (with the exception of rats and mice) have shown that the arterial wall contains by far the greatest content of SOD of all the organs tested [3], suggesting an important role for the enzyme in this tissue.

Recently an in vitro model system developed in this laboratory has shown that immunoglobulin molecules have an associated SOD activity with a pH maximum of 6.45 at which classic Cu/Zn-SOD activity is much reduced (typically to < 10 % of that at pH 7.81) [4]. This finding has led us to speculate that the known accumulation of IgG within the aortic intima during progression of atherosclerosis [5] may result in a degree of auto-protection of tissues from the oxidative damage which occurs during atherosclerosis. With this in mind the superoxide dismutase (SOD) activity of human aortic intima has been measured on the basis of H_2O_2 production from superoxide in PBS extracts of matched normal and advanced lesion pairs from 19 patients aged 56-92 years, (Table 1).

### Table 1: SOD activity of PBS extracts of normal and lesion

<table>
<thead>
<tr>
<th>DISEASE STATUS</th>
<th>SOD activity at pH 7.81 (micromol H_2O_2/mg protein/min)</th>
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</thead>
<tbody>
<tr>
<td>REAGENT</td>
<td>+ DDC</td>
</tr>
<tr>
<td>Normal</td>
<td>2.54 ± 0.07</td>
</tr>
<tr>
<td>Lesion</td>
<td>2.94 ± 0.75</td>
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<table>
<thead>
<tr>
<th>DISEASE STATUS</th>
<th>SOD activity at pH 6.45 (micromol H_2O_2/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REAGENT</td>
<td>+ DDC</td>
</tr>
<tr>
<td>Normal</td>
<td>0.87 ± 0.11</td>
</tr>
<tr>
<td>Lesion</td>
<td>3.92 ± 0.09</td>
</tr>
</tbody>
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At pH 7.81, SOD activity in the lesion is twice that in normal artery. Reduction of the pH of the assay to pH 6.45 did not reduce the specific Cu/Zn-SOD activity in the lesion extracts, but reduced the activity of normal tissue extracts by approximately half, suggesting that Cu/Zn-SOD contributes a greater percentage of total activity to the normal than lesion tissue. Immunoprecipitation of tissue extracts with Staphylococcal protein A, which specifically binds antibody molecules [7], removed approximately 50% of the activity from lesion extracts at pH 7.8, and > 70 % of the activity at pH 6.45, but had no effect on extracts from normal tissue suggesting that most of the activity could not be attributed to classic CuZn-SOD and may be due to accumulated antibodies which have SOD-like activity (IgG-SOD). The effect of the CuZn-SOD inhibitor, diethyldithiocarbamic acid (DDC) on these samples is somewhat harder to interpret. This compound has high binding affinity for Cu (II) ions and is a potent inhibitor of CuZn-SOD activity (> 80% inhibition), but has a variable inhibitory action on Ig-SOD activity, which may be affected by the Cu (II) content of these molecules. Since DDC appears to inhibit SOD activity of lesion-derived Ig, it may be that some lesion-Igs contain bound Cu (II), which has been reported to exist in a free form in some lesions [8], and enhances the catalytic activity of Ig molecules in other systems [9].

To show whether specific domains of IgGs are responsible for Ig-SOD activity, Fab regions and Fc regions can be split from parent IgG and fragments purified and assayed [4]. Initial results suggest that >30% activity is located in the Fab region of IgG, as predicted by the current model, though further activity may also be associated with the Fc region (currently untested). Although there is no obvious sequence similarity between IgG and CuZn-SOD, the three dimensional shape of SOD resembles that of the Fab region of antibodies which all share in common a Greek key structure of alternating beta-sheet [10], and this may provide the framework for the SOD activity of members of the immunoglobulin superfamily.

The high SOD content of the human arterial wall deserves attention in studies on vascular wall pathology which potentially involve oxidative stress. The SOD activity of immunoglobulins may be a hitherto missing link between immune processes and diseases where oxygen centred free radicals are thought to play a role. Since the pH on cell surfaces and within the atherosclerotic lesion has been proposed to be acidic [11] it is possible that IgG SOD activity is biologically important in both normal and pathological conditions. It is also conceivable that antibody-mediated reactions might serve to deliver SOD activity to sites of tissue damage and thus contribute to limitation of damage.

Acknowledgements

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References: