Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin

D Roy, J Quiles, D C Gaze, P Collinson, J C Kaski, G F Baxter

Role of reactive oxygen species (ROS) such as superoxide (‘O$_2^-$’ and hydroxyl (‘OH’) radicals generated during myocardial ischaemia–reperfusion modify the N-terminus of serum albumin resulting in IMA formation but, so far, direct evidence to support this is scarce. We hypothesised that ROS generation causes the formation of IMA. Our objective was to model the formation of IMA in vitro by using chemically generated ROS and the ‘OH radical scavenger mercaptopropionylglycine (MPG).

MATERIALS AND METHODS

All reagents were obtained from Sigma-Aldrich (Poole, Dorset, UK) and normal human serum from the North London Transfusion Service (London, UK). Serum pH was 7.35–7.45 at 37°C and was unaltered by any of the reagents during the 15 minute experimental time course. Five millilitre serum aliquots (eight replicate experiments per group) were randomly selected for the following incubations for 15 minutes at 37°C. Group 1, peroxide treated (H$_2$O$_2$): serum was incubated with H$_2$O$_2$ 100 μM, Group 2, superoxide treated (‘O$_2^-$’): serum was incubated with a xanthine-xanthine oxidase ‘O$_2^-$’ generating system consisting of 100 μM xanthine plus 0.05 U/ml xanthine oxidase (xanthine → urate$^+$ + ‘O$_2^-$’). Group 3, hydroxyl treated (‘OH’): ‘OH was generated by the Fenton reaction and Cu$^{2+}$ 0.1 mmol/l catalysed the generation of ‘OH from H$_2$O$_2$ 100 μM (H$_2$O$_2$ + Cu$^+$ → ‘OH + OH$^-$ + Cu$^{2+}$). Group 4, Cu$^{2+}$ control: aliquots of serum were incubated with 0.1 mM CuSO$_4$ as a technical control for group 3. Group 5, ‘OH + MPG treated: aliquots of serum were incubated with the ‘OH generating mixture (as in group 3) with the addition of MPG 1 mM. Group 6, control: serum was incubated for 15 minutes without the addition of any reagents.

Samples were withdrawn for analysis of IMA at baseline and after 2, 5, 10, and 15 minutes’ incubation. Samples were frozen at -70°C for blinded IMA determination by the ACB test (ACB Test, Ischemia Technologies Inc, Denver Colorado, USA) on a Roche Cobas MIRA PLUS analyser (ABX Ltd, London, UK). The principle of the test is as follows. Co$^{2+}$ is added to serum. Co$^{2+}$ not sequestered at the N-terminus of albumin is detected by dithiothreitol as a colorimetric indicator. In normal serum, more Co$^{2+}$ is sequestered at the N-terminus of albumin, leaving less Co$^{2+}$ to react to form a coloured product. After chemical modification of serum, Co$^{2+}$ is not sequestered at the N-terminus of albumin, leaving more free Co$^{2+}$ to react. The total interassay coefficient of variation was 4.9–7.5% at 72.54–140.16 U/ml for quality control material. For human serum pools, the total coefficient of variation was 5.3–8.8% at 95.07–97.35 U/ml.

The percentage change in IMA in each group was calculated as follows: (IMA concentration at each time point—baseline IMA concentrations)/baseline IMA concentration × 100. Results are expressed as mean (SE) of eight replicate experiments. IMA concentrations for each treatment were time matched by repeated measures analysis of variance with Bonferroni post hoc comparisons by SPSS 11.0 statistical software (SPSS Inc, Chicago, Illinois, USA). Results were considered significant when p < 0.05.

RESULTS

Table 1 presents changes in IMA concentration. In control serum (group 6) IMA concentrations did not change significantly at any time point. Neither H$_2$O$_2$ (group 1) nor ‘O$_2^-$’ (group 2) caused any significant change in IMA concentration during the experimental time course compared with control. However, generation of ‘OH by the Fenton reaction (group 3) was associated with a rapid rise of IMA concentration. A maximum increase of 43.6% greater than baseline was observed at 15 minutes. The addition of the ‘OH scavenger MPG (group 5) to the Fenton reaction mixture attenuated the production of IMA (no significant difference versus control group 6).

Separate titrimetric analysis confirmed that H$_2$O$_2$ concentration was reduced by 22.0 (3.0)% (four determinations) during 15 minutes’ incubation as a result of serum catalase activity. Serum albumin concentration, determined by immunonephelometric assay, was 42.4 g/l before treatment and was not altered by 15 minutes’ incubation with any of the reactants studied.

Since Cu$^{2+}$ may interfere with the Co$^{2+}$ binding assay, an important technical control in our experiments was group 4. We observed that Cu$^{2+}$ 0.1 mM in the absence of ‘OH generation resulted in no significant change in IMA concentrations. Theoretically, Cu$^{2+}$ bound to the N-terminus prevents Co$^{2+}$ binding because the binding constant for Cu$^{2+}$ (K$\text{Cu}^{2+} = 1.5 \times 10^{16}$ mol/l) is many orders of magnitude higher than that for Co$^{2+}$ (K$\text{Co}^{2+} = 6.5 \times 10^1$ mol/l). However, in these experiments we observed no appreciable interference of Cu$^{2+}$.

DISCUSSION

In vivo modifications of the albumin N-terminus are proposed to be related to the ROS production during myocardial ischaemia–reperfusion. For example, an increase in IMA was observed in patients minutes after transient occlusion and reperfusion during coronary angioplasty.

Abbreviations: ACB, albumin Co$^{2+}$ binding; IMA, ischaemia modified albumin; MPG, mercaptopropionylglycine; ROS, reactive oxygen species; SOD, superoxide dismutase
present in vitro investigation shows for the first time that
IMA formation is directly related to ROS generation and
provides novel information on the nature of the species
contributing to IMA formation, namely 'OH.

Previous studies in vitro have shown that the exposure of
albumin to 'OH generated by copper (at similar concentra-
tions to those used in our experimental series) and ascorbate
resulted in modification of albumin in a site specific manner,
rather than generalised degradation.4 ROS generation in vitro
causcd structural changes in a synthetic N-terminus tetra-
peptide, an octapeptide, and human albumin with loss of
caused partial degradation of H2O2 (22%) during
5 minutes' incubation. Thus, the H2O2 concentration
remained high (>70 μM) throughout the experimental time
course. The dismutation of 'O2- by extracellular superoxide
dismutase (SOD) present in serum undoubtedly occurs in
vitro, just as it does in vivo. Moreover, the presence of
intracellular SOD isoenzymes in vivo may further reduce the
availability of 'O2-. At present, we cannot say whether the
efficiency of extracellular SOD in accomplishing the dis-
mutation of 'O2- accounts for the lack of effect of 'O2 or
whether 'O2- has low chemical reactivity with albumin in
vitro. It has been suggested that 'O2- per se is not deleterious
but serves as a source of highly reactive secondary species
such as 'OH, which are responsible for biological damage.

These data support the hypothesis that ROS, and specifi-
cally in our hands 'OH, may chemically modify human serum
albumin, resulting in IMA formation. This appears to be a
plausible mechanism underlying this new diagnostic test. To
improve the clinical utility of IMA a clear understanding of
the mechanism of the reaction relevant to in vivo ischaemia–
reperfusion is essential. Clearly, further in vivo studies
supported by data from clinical trials are required to confirm
the novel mechanism we have identified in these in vitro
studies. Moreover, it will be important to elucidate the
anatomical sites of IMA formation and its value in the
diagnosis of disease states associated with oxidative stress,
including myocardial ischaemia.

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REFERENCES
1 Morrow DA, De Lemos JA, Sabatine MS, et al. The search for a biomarker of
2 Sinha M, Gaze DC, Tippins JR, et al. Ischemia-modified albumin is a sensitive
marker of myocardial ischemia after percutaneous coronary intervention.
3 Bar-Or D, Winkler JV, Vanbenthuyzen K, et al. Reduced albumin-cobalt
binding with transient myocardial ischemia after elective percutaneous
transluminal coronary angioplasty: a preliminary comparison to creatine
4 Sinha MK, Roy D, Gaze DC, et al. Role of "ischemia-modified albumin", a
new biochemical marker of myocardial ischemia, in the early diagnosis of
5 Marx G, Chevian M. Site-specific modification of albumin by free radicals:
reaction with copper(II) and ascorbate. Biochem J 1986;236:397–400.
6 Chan B, Dodssworth N, Woodrow J, et al. Site-specific N-terminal auto-

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**Table 1** Ischaemia modified albumin percentage change from baseline concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Incubation time (min)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>1: H2O2</td>
<td>–0.7 (1.0)</td>
<td>–7.1 (4.3)</td>
</tr>
<tr>
<td>2: 'O2-</td>
<td>3.9 (2.4)</td>
<td>5.5 (3.0)</td>
</tr>
<tr>
<td>3: 'OH</td>
<td>28.3 (3.4)</td>
<td>34.7 (3.6)</td>
</tr>
<tr>
<td>4: Cu2+ control</td>
<td>4.1 (1.4)</td>
<td>6.4 (0.7)</td>
</tr>
<tr>
<td>5: 'OH + MPG</td>
<td>6.2 (8.5)</td>
<td>6.1 (8.7)</td>
</tr>
<tr>
<td>6: control</td>
<td>0.7 (1.5)</td>
<td>–0.5 (0.8)</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SEM) of eight replicate experiments. p Values relate to comparison with group 6 (control).
NS, not significant (repeated measures analysis of variance with Bonferroni post hoc test).
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