Oxygen-regulated protein 150 and prognosis following myocardial infarction

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ABSTRACT

ORP150 (oxygen-regulated protein 150) is a chaperonin expressed in tissues undergoing hypoxic or endoplasmic reticulum stress. In the present study, we investigated plasma levels of ORP150 in patients with AMI (acute myocardial infarction) and its relationship with prognosis, together with a known risk marker N-BNP (N-terminal pro-B-type natriuretic peptide). Plasma from 396 consecutive patients with AMI was obtained for measurement of ORP150 and N-BNP. Mortality and cardiovascular morbidity (acute coronary syndromes/heart failure) was determined during follow-up. A specific ORP150 assay detected the 150 kDa protein in plasma extracts, including 3 and 7 kDa fragments. During follow-up (median, 455 days), 43 (10.9%) patients died. Both N-BNP and ORP150 levels were higher in those who died compared with the survivors [N-BNP, 724 (14.5–28 840) compared with 6167 (154.9–33 884) pmol/l (P < 0.0005); ORP150, 257 (5.9–870.9) compared with 331 (93.3–831.8) pmol/l (P < 0.001); values are medians (range)]. In a Cox regression model for mortality prediction, both N-BNP (odds ratio, 5.06; P < 0.001) and ORP150 (odds ratio, 2.39; P < 0.01) added prognostic information beyond creatinine and the use of thrombolytics. A Kaplan–Meier survival analysis revealed that ORP150 added prognostic information to N-BNP, especially in those with supra-median N-BNP levels. A simplified dual-marker approach with both markers below and either above or both above their respective medians effectively stratified mortality risk (log rank statistic for trend, 32.7; P < 0.00005). ORP150 levels were not predictive of other cardiovascular morbidity (acute coronary syndromes or heart failure). In conclusion, ORP150 and peptide fragments derived from it are secreted following AMI and provide independent prognostic information on mortality. High levels associated with endoplasmic reticulum/hypoxic stress predict a poor outcome.

INTRODUCTION

The prognosis following AMI (acute myocardial infarction) varies widely, and recent evidence suggests that biochemical markers may be suitable for risk stratification. In particular, plasma levels of BNP (B-type natriuretic peptide) or N-BNP (N-terminal pro-BNP) obtained in the sub-acute phase have proved to be useful in predicting mortality [1,2]. These peptides may reflect changes in wall stress following AMI. An additional biochemical marker which reflects tissue hypoxia may confer improvements in risk assessment.

Recent work on tissue hypoxia and endoplasmic reticulum stress has led to the cloning of a stress protein called

Key words: atherosclerosis, coronary disease, hypoxia/anoxia, myocardial infarction, natriuretic peptide, oxygen-regulated protein 150 (ORP150).

Abbreviations: AMI, acute myocardial infarction; BNP, B-type natriuretic peptide; CK, creatine kinase; ILMA, immunoluminometric assay; N-BNP, N-terminal pro-BNP; ORP150, oxygen-regulated protein 150; ROC, receiver operating characteristic.

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ORP150 (oxygen-regulated protein 150), a 150 kDa endoplasmic-reticulum-associated protein that functions as a chaperone for protein folding and maturation [3]. The induction of this protein in rat astrocytes, human aortic vascular myocytes and mononuclear leucocytes showed specificity for hypoxia, but not other stressful stimuli [3,4]. In addition, tissue extracts prepared from human atherosclerotic lesions had an increased expression of ORP150 mRNA and protein, with most of the mRNA found in macrophages [4]. Its functions include a protective effect against hypoxia-induced damage and endoplasmic reticulum stress, since reducing its expression with antisense oligonucleotides leads to enhanced susceptibility of mononuclear phagocytes to hypoxic damage [4]. Evidence for a function of ORP150 in vascular disease comes from observations in a stroke model in mice [5,6]. In a mouse model of cerebral ischaemia, there was a rapid induction of ORP150 mRNA and protein in the hypoxic neurons, even within the ischaemic and energy-depleted zones [5]. In ischaemic human brains, although ORP150 expression in neurons was only sparingly induced, there was a significant induction of ORP150 in astrocytes [6]. Neurons overexpressing ORP150 were resistant to hypoxic stress, and mice genetically engineered to overexpress ORP150 in their neurons had smaller strokes under ischaemic stress [6]. Cytoprotection was associated with suppressed caspase-3-like activity and enhanced BDNF (brain-derived neurotrophic factor), indicating a role for ORP150 in cytoprotection under hypoxic conditions [6]. In cultured rat cardiomyocytes, hypoxia/re-oxygenation-induced cell death was enhanced or attenuated by reduced or increased ORP150 expression respectively, within the cells [7]. Decreased ORP150 expression was associated with enhanced caspase 3 and 8 activation, cytochrome c release and DNA fragmentation, reflecting increased apoptotic cell death. Increased ORP150 expression in rat hearts led to improved functional recovery after ischaemia [7]. There is also a suggestion that ORP150 may be secreted in diabetes, reflected in an increased autoantibody titre to ORP150 [8].

In the present study, we investigated whether ORP150 was present in human plasma after cardiac tissue hypoxia (AMI), and whether its measurement following AMI improves the risk stratification provided by N-BNP, a recognized risk marker.

**MATERIALS AND METHODS**

**Study population**

We studied 396 consecutive patients admitted to the Coronary Care Unit of Leicester Royal Infirmary with AMI between September 1999 and April 2001. AMI was defined as presentation with at least two of three standard criteria, i.e. appropriate symptoms, acute ECG changes of infarction (ST elevation and new left bundle branch block) and a rise in CK (creatine kinase) to at least twice the upper limit of normal, i.e. > 400 international units/l. Relevant clinical information, such as prior medical history, pharmacological therapy, Killip class, cardiac enzymes, renal function and lipid profile, was collected. All patients were followed-up and end points of all-cause mortality or cardiovascular morbidity (such as further hospitalization with acute coronary syndromes, heart failure or revascularization) were validated by a review of hospital notes. Normal subjects [n = 125 (95 male); age, 64.2 ± 7.5 years (mean ± S.D.)] were recruited from the community and had no symptoms, signs, echocardiographic or ECG evidence of heart disease and were on no medication. All subjects gave informed consent to participation in the study, which was approved by the local Ethics Committee. The study conforms to the principles of the Declaration of Helsinki.

**Blood sampling**

A single blood sample for measurement of plasma ORP150 and N-BNP was taken 72–96 h after symptom onset, based on previous work from our group [2]. A total of 20 ml of peripheral venous blood was drawn into pre-chilled EDTA (1.5 mg/ml blood) tubes containing 500 international units/ml aprotinin. Samples were centrifuged at 1500 g at 4°C for 15 min, before the plasma was separated and stored at −70°C until assay.

**Assay for ORP150 and N-BNP**

N-BNP was measured in 10–20 µl of unextracted plasma using a validated in-house immunochemiluminometric assay, as previously described [2]. The ORP150 assay was a competitive assay constructed using a polyclonal antibody raised in rabbits immunized with keyhole limpet haemocyanin conjugated with a peptide corresponding to the N-terminal domain (amino acids 33–45) of the human ORP150 sequence (LAVMSVDLGSEM). IgG from the sera was purified on Protein A-Sepharose columns and then affinity-purified on an Affigel column with an immobilized peptide. The biotinylated peptide tracer was purified by reverse-phase HPLC using an acetonitrile gradient. Prior to assay, plasma was extracted on C18 Sep-Pak columns and dried on a centrifugal evaporator. Plasma extracts and standards were reconstituted with ILMA (immunoluminometric assay) buffer consisting of 1.5 mmol/l NaH2PO4, 8 mmol/l Na2HPO4, 140 mmol/l NaCl, 1 mmol/l EDTA, 1 g/l BSA and 0.1 g/l azide (pH 7.0). ELISA plates were coated with 100 ng of anti-rabbit IgG (Sigma) in 100 µl of 0.1 mol/l sodium bicarbonate buffer (pH 9.6). Wells were then blocked with 0.5% BSA in bicarbonate buffer. A competitive ILMA was set up by pre-incubating 20 ng of the IgG with standards or samples within the wells. After overnight incubation, 50 µl of the diluted biotinylated ORP150 peptide tracer (100 fmol) was added...
to the wells. Following another 24 h of incubation at 4°C, wells were washed three times with a wash buffer [1.5 mmol/l NaH₂PO₄, 8 mmol/l Na₂HPO₄, 340 mmol/l NaCl, 0.5 g/l Tween 20 and 0.1 g/l sodium azide (pH 7.0)]. Detection was with streptavidin labelled with methyl-acridinium ester, as described previously [2]. Within-assay coefficients of variation were 3.1, 4.3 and 5.9% for 2, 30 and 500 fmol/tube respectively. There was no cross-reactivity with peptides demonstrated previously to be elevated in heart failure, such as ANP (atrial natriuretic peptide), BNP, N-BNP or CNP (C-type natriuretic peptide). Markers of AMI, such as troponin I, troponin T or CK, would not be extracted by C₁₈ Sep-Pak columns due to their large molecular masses. In addition, neither CK-MB (35 ng/ml) nor troponin I (50 ng/ml) was detectable in the ORP150 assay. These levels of CK-MB and troponin I are similar to those described in AMI after 3–5 days [9].

Size-exclusion chromatography and gel electrophoresis of plasma extracts

Plasma extracts were fractionated by isotropic size-exclusion chromatography on a 300 × 7.8 mm Bio-Sep SEC S2000 column (Phenomenex) using 50 mmol/l NaH₂PO₄ (pH 6.8) at a flow rate of 1 ml/min as the mobile phase. Standards used to establish molecular masses included IgG (150 kDa), BSA (68 kDa), ovalbumin (44 kDa), soybean trypsin inhibitor (20 kDa), aprotinin (6.5 kDa) and tryptophan (204 Da) (Sigma). Fractions collected every 20 s were dried on a centrifugal evaporator before being assayed for ORP150 as above.

Western blotting of cell extracts

GM-1 monocytic cells (Roche Pharma; [10]) were cultured in RPMI growth medium (containing 10% fetal calf serum). The GM-1 cell line is derived from the human U937 monocyte cell line and can be differentiated using 1 µmol/l retinoic acid, 10 mmol/l 1,25-dihydroxycholecalciferol and 200 units/ml interferon-γ (all from Sigma). Following 4 days of culture with these differentiation-inducing agents, GM-1 cells were treated with 50 µmol/l H₂O₂ for 3 h, as this has been reported to up-regulate ORP150 expression [11]. Cells were extracted in Laemli sample buffer, reduced with dithiothreitol, and protein samples (100 µg) were resolved on SDS/10% (w/v) polyacrylamide gels. Following Western blotting, nitrocellulose membranes were blocked overnight in 10% (w/v) non-fat milk/Tris-buffered saline (pH 7.4) containing 0.1% Tween 20. Blots were incubated with 1 µg/ml anti-ORP150 antibodies [our in-house rabbit polyclonal antibody and a commercial monoclonal mouse ORP150 IgG2a antibody (clone 2F07; Immuno-Biological Laboratories), which was raised against a recombinant protein containing amino acids 508–999 of the ORP150 sequence] for 2 h. The specificity of the Immuno-Biological Laboratories monoclonal antibody has been confirmed in ORP150 transgenic and knockout mice (http://www.ibl-japan.co.jp/en.goods/pamphlet/ORP150_Pamph_English.pdf). The secondary antibodies used were HRP (horseradish peroxidase)-conjugated goat anti-(rabbit IgG) or anti-(mouse IgG) antibodies respectively. Detection was by enhanced chemiluminescence.

Statistical analysis

Data were analysed by SPSS Version 10.1 and are presented as means (S.D.) or medians (range) for data with non-Gaussian distribution, which were log-transformed prior to analysis. For continuous variables, mean levels in normal subjects and those suffering or being spared each clinical event were compared by ANOVA with Bonferroni correction for multiple comparisons, or the Kruskal–Wallis test (for non-parametric analyses). Mann–Whitney P values are reported for non-parametric analyses. Clinical variables incorporated as potential predictors of outcome included age, Killip class (dichotomized as class I compared with classes II, III or IV), log plasma creatinine and history of prior AMI, heart failure, hypertension or diabetes. The independent predictive power of peptide levels and clinical factors was tested using Cox proportional hazards regression, using both forward and backward likelihood-ratio analysis. Kaplan–Meier survival curves were constructed for groups with ORP150 or N-BNP above and below their respective median values, and the log rank statistic for the trend with associated P values are quoted. Two-tailed P values of < 0.05 were considered significant.

RESULTS

A Western blot of GM-1 cell extracts is shown in Figure 1(A). Both the commercial ORP150 monoclonal antibody and our in-house rabbit polyclonal affinity-purified antibody detected a single band of molecular mass of approx. 147 kDa, which resembles the reported molecular mass of ORP150 [3, 4].

A typical standard curve for the ORP150 peptide is illustrated in Figure 1(B), showing a fall in chemiluminescence with increasing concentrations of the peptide. Dilutions of plasma extracts from patients confirmed parallelism with the standard curve. Isotric size-exclusion chromatography of human plasma extracts confirmed the presence of the 150 kDa protein (the predicted molecular mass of mature ORP150 protein), as well as two other immunoreactive fractions at approx. 3 and 7 kDa (Figure 2), which are likely to be degradation products consisting of the N-terminal of ORP150 (the epitope to which the antiserum was directed).

ORP150 was detectable in the extracted plasma from 125 normal subjects, who were age- and gender-matched...
Figure 1  Representative Western blot of GM-1 cell extracts (A), and a standard curve for the ORP150 peptide competitive immunoassay (B)

(A) Both the in-house rabbit polyclonal antibody and the Immuno-Biological Laboratories (IBL) ORP150 monoclonal antibody detected a single band of molecular mass of approx. 147 kDa. (B) Plasma extracts from three patients (●, △ and ▲) were diluted in 2-fold steps, showing parallelism with the standard curve (○).

Figure 2  Size-exclusion chromatography with analysis of the fractions for ORP150

The elution of markers at 150 kDa (IgG), 20 kDa (soybean trypsin inhibitor) and 6.5 kDa (aprotinin) are indicated by arrows. Three peaks of ORP150 immuno-reactivity were evident at 150 kDa, approx. 7 kDa and approx. 3 kDa.

Figure 3  Box-and-whisker plots showing plasma N-BNP and ORP150 levels in normal subjects, survivors post-AMI and patients who died post-AMI

Boxes represent interquartile ranges, with the median level marked. The whiskers represent the 97.5 percentiles of the observed values.

with the patients with AMI. The median (range) ORP150 level was 138 (13–562) pmol/l and N-BNP level was 22.4 (5.7–631) pmol/l (Figure 3). In normal subjects, ORP150 was not correlated with age, although there was a weak correlation with plasma creatinine ($r_s = 0.212, P < 0.02$).

Table 1 shows the clinical characteristics of the patients. Patients who died ($n = 43$) during the median follow-up period of 455 days were older, had higher plasma creatinine levels and a higher incidence of previous AMI. No patient was lost to follow-up. Those who died did not have a higher prevalence of anterior AMI, although they had a lower thrombolytic treatment rate ($P < 0.0005$ as determined by $\chi^2$ test). Plasma levels of N-BNP [851 (14.5–33884) pmol/l] and ORP150 [266 (5.9–870.9) pmol/l] were significantly higher ($P < 0.001$ for both) in the patients post-AMI compared with controls (Figure 3). Plasma levels of both N-BNP and ORP150 were significantly higher in patients post-AMI who died compared with survivors ($P < 0.0005$ and $P < 0.004$ for N-BNP and ORP150 respectively, as determined
Table 1 Clinical details of the patients post-AMI
Values are medians (range) or n (%). P values were determined by Mann–Whitney, Kruskal–Wallis or χ² tests and are comparisons between the patients who died and those who survived.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Patients with AMI who died</th>
<th>Patients with AMI who survived</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>396</td>
<td>43</td>
<td>353</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.5 (32–95)</td>
<td>73 (55–89)</td>
<td>65 (32–95)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Male (n)</td>
<td>295 (74.5%)</td>
<td>33 (76.7%)</td>
<td>262 (74.2%)</td>
<td>0.720</td>
</tr>
<tr>
<td>Follow-up (days)</td>
<td>465 (5–764)</td>
<td>67 (5–619)</td>
<td>479 (179–764)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Anterior AMI (n)</td>
<td>143 (101)</td>
<td>18 (7)</td>
<td>125 (94)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Other site AMI (n)</td>
<td>253 (164)</td>
<td>25 (8)</td>
<td>228 (156)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Previous history (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td>78 (19.7%)</td>
<td>17 (39.5%)</td>
<td>61 (17.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>134 (33.8%)</td>
<td>19 (44.2%)</td>
<td>115 (32.6%)</td>
<td>0.129</td>
</tr>
<tr>
<td>Diabetes</td>
<td>88 (22.2%)</td>
<td>13 (30.2%)</td>
<td>75 (21.3%)</td>
<td>0.181</td>
</tr>
<tr>
<td>Heart failure</td>
<td>20 (5.1%)</td>
<td>4 (9.3%)</td>
<td>16 (4.5%)</td>
<td>0.177</td>
</tr>
<tr>
<td>Highest Killip class (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>172 (43.7%)</td>
<td>9 (21.4%)</td>
<td>163 (46.3%)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>II</td>
<td>160 (40.6%)</td>
<td>14 (33.3%)</td>
<td>146 (41.5%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>53 (13.5%)</td>
<td>15 (35.7%)</td>
<td>38 (10.8%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>4 (9.5%)</td>
<td>4 (9.5%)</td>
<td>5 (1.42%)</td>
<td></td>
</tr>
<tr>
<td>Plasma measurements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>96 (35–346)</td>
<td>128 (61–346)</td>
<td>94 (35–265)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Peak CK (m-units/l)</td>
<td>1371 (75–15 456)</td>
<td>1191 (312–12 652)</td>
<td>1394 (75–15 456)</td>
<td>0.419</td>
</tr>
<tr>
<td>N-BNP (pmol/l)</td>
<td>851 (14.5–33 884)</td>
<td>6167 (154.9–33 884)</td>
<td>724 (14.5–28 840)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>ORP150 (pmol/l)</td>
<td>266.1 (5.9–870.9)</td>
<td>331 (93.3–831.8)</td>
<td>257 (5.9–870.9)</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

by ANOVA with Bonferroni correction; Table 1 and Figure 3). There was no difference in ORP150 levels in patients with a previous history of AMI, diabetes, heart failure or hypertension.

ROC (receiver operating characteristic) curves for N-BNP and ORP150 in prediction of all-cause mortality are shown in Figure 4, the area under the curves being 0.808 (95% confidence interval, 0.736–0.880) and 0.661 (95% confidence interval, 0.575–0.747) respectively. The correlation between ORP150 and N-BNP (rₛ = 0.149, P < 0.003) or plasma creatinine (rₛ = 0.171, P < 0.001) was modest, with no significant correlation with age. Although plasma N-BNP was correlated with peak plasma CK levels (rₛ = 0.226, P < 0.0005), ORP150 had no such correlation (rₛ = 0.02, P = not significant). ORP150 levels did not differ according to gender or site of infarction.

Patients with Killip class > I had slightly higher ORP150 levels than those with Killip class I, which did not achieve conventional levels of significance [(281.8 (5.9–870.9) compared with 251.2 (8.9–831.8) pmol/l; P < 0.07). There were no significant differences in ORP150 levels between those patients with Killip classes II, III or IV. This contrasts with N-BNP, where higher levels were demonstrated: (i) in patients with median age > 67 years [1412 (14.5–33 884) pmol/l compared with 543 (14.5–14 125) pmol/l in patients with median age ≤ 67 years; P < 0.0005], (ii) in females [1445 (35.5–33 884) pmol/l compared with 691 (14.5–28 840) pmol/l

Figure 4 ROC curves for N-BNP and ORP150 in the prediction of all-cause mortality

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Figure 5  Kaplan–Meier survival analysis of patients following AMI, stratifying patients as below or above the median values of plasma N-BNP or ORP150

Median value of N-BNP, 851 pmol/l; median value of ORP150, 266 pmol/l.

in males; P < 0.0005], (iii) in those with anterior AMI [1096 (14.5–16982) pmol/l compared with 692 (14.5–33 884) pmol/l in patients with the other site of AMI; P < 0.02], and (iv) in those with Killip class > I [1244 (14.5–33 884) pmol/l compared with 513 (14.5–21 877) pmol/l in patients with Killip class = I; P < 0.0005].

Plasma N-BNP, ORP150 and all-cause mortality

Kaplan–Meier survival curves for supra- and infra-median peptide levels for N-BNP (median value, 851 pmol/l) or ORP150 (median value, 266 pmol/l) are shown in Figure 5. Both plasma markers were significant predictors of mortality [log rank χ² statistic, 29.53 for N-BNP (P < 0.00005) and 8.62 for ORP150 (P < 0.003)]. The predictive value of ORP150 was examined in patients stratified for N-BNP levels (Figure 6). The log rank χ² statistic for supra-compared with infra-median ORP150 levels adjusted for supra-compared with infra-median N-BNP levels was 6.98 (P < 0.008 for trend). However, the incremental value of ORP150 was most evident in the group with supra-median N-BNP levels > 851 pmol/l (log rank χ² statistic, 5.26; P < 0.02). ORP150 levels provided no incremental information on survival in those patients with infra-median N-BNP values (log rank χ² statistic, 2.04; P = not significant).

The unadjusted odds ratios for mortality were 9.45 for N-BNP and 2.88 for ORP150. Using stepwise forward likelihood ratio logistic regression analysis for prediction of all-cause mortality with supra- and infra-median levels of ORP150, N-BNP, log creatinine, age, previous history of AMI, anterior site of AMI, use of thrombolysis and peak CK levels, a model with ORP150, N-BNP, log creatinine and use of thrombolysis as the only independent predictive factors accounted for a Nagelkerke r² of 0.33 (P < 0.0005). Odds ratios were 2.98 (P < 0.006), 5.87 (P < 0.001), 61.29 (P < 0.001) and 0.25 (P < 0.0005) for supra-compared with infra-median values of ORP150, N-BNP, log creatinine and use of thrombolysis respectively. Killip class was not an independent predictor of death.

Cox proportional hazard modelling was employed to adjust for potential confounding factors in the prediction of mortality. Table 2 shows that supra-compared with infra-median ORP150 levels maintained an independent predictive value, together with supra-compared with
Table 2  Cox regression model for survival post-AMI

<table>
<thead>
<tr>
<th>Variable</th>
<th>B</th>
<th>S.E.M.</th>
<th>P value</th>
<th>Hazard ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-BNP &gt; 851 pmol/l</td>
<td>1.622</td>
<td>0.492</td>
<td>0.001</td>
<td>5.06</td>
</tr>
<tr>
<td>ORP150 &gt; 1266 pmol/l</td>
<td>0.87</td>
<td>0.345</td>
<td>0.01</td>
<td>2.39</td>
</tr>
<tr>
<td>Log plasma creatinine</td>
<td>3.541</td>
<td>0.931</td>
<td>0.001</td>
<td>34.50</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>1.224</td>
<td>0.324</td>
<td>0.001</td>
<td>0.29</td>
</tr>
</tbody>
</table>

DISCUSSION

ORP150 was initially described as a chaperone protein localized to the endoplasmic reticulum with a role in protein folding and maturation [6]. Suppression of its expression led to larger cerebral infarct size [6] and enhanced susceptibility to hypoxic damage [4,7]. The specificity of our in-house antibody was similar to that of a commercially available ORP150 monoclonal antibody, which had been validated in transgenic and knockout mice models. Although the ORP150 protein is predominantly intracellular, damage to plasma membranes during AMI may permit leakage of proteins to the extracellular space. Thus tissue ORP150 expression may be reflected in plasma levels, which we describe for the first time in the present study. These levels may, in turn, reflect endoplasmic reticulum stress in hypoxic tissues.

Our present data suggests that ORP150 in plasma was not solely the 150 kDa protein, but smaller fragments containing the N-terminal were also present. Fragmentation of the protein could have occurred from endoproteases present in necrotic tissue.

The levels of ORP150 following AMI were weakly correlated with N-BNP, and both were higher in those who died during follow-up compared with survivors. However, the factors that affected plasma N-BNP had little influence on ORP150 levels (for example, Killip class, site of infarction, age, creatine kinase levels and gender). Thus N-BNP and ORP150 may reflect different processes in the pathophysiology of AMI, with N-BNP reflecting wall stress and ORP150 reflecting endoplasmic reticulum stress. This was confirmed by the independent predictive value for death of ORP150 when used in conjunction with N-BNP in Cox proportional hazards analysis and in binary logistic regression analysis. From the physiology of the natriuretic peptide and ORP150 systems, they are likely to be counter-regulatory to ischaemic damage, and the relationship with a poor prognosis may reflect the degree of tissue damage. However, a deleterious effect of excessive ORP150 expression cannot be discounted, as recent evidence suggests that transgenic mice overexpressing this protein suffer from age-related vacuolar degeneration of cardiac myocytes [12].

When patients were stratified for N-BNP levels, it was evident that ORP150 had an incremental value in defining mortality risk in those with supra-median N-BNP levels, enabling even higher risk patients to be distinguished. Thus those patients with both markers below the median have a good prognosis compared with those with both markers above the median. The effective identification of these high-risk patients early in the course of AMI may facilitate the focusing of therapy also no relationship with the development of angina or positive exercise tests following AMI.
towards this group (whether pharmacological, inter-
ventional or device-related).

A limitation of our present study is the uncertainty concerning whether modulating endoplasmic reticulum stress and ORP150 following AMI could influence the poor outcome, and this should be the subject of further research.

Conclusions
This is the first report of a relationship between high plasma levels of a chaperone hypoxic stress-related pro-
tein (ORP150) following AMI and increased mortality. ORP150 has incremental predictive value for all-cause mortality over an accepted risk marker such as N-BNP. Endoplasmic reticulum stress following AMI may indicate a poor prognosis.

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REFERENCES

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