Intravenous Selenium Modulates L-Arginine-Induced Experimental Acute Pancreatitis

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ABSTRACT

Context Oxidative stress is understood to have a critical role in the development of acinar injury in experimental acute pancreatitis. We have previously demonstrated that compound multiple antioxidant therapy ameliorates end-organ damage in the intra-peritoneal L-arginine rat model. As the principal co-factor for glutathione, selenium is a key constituent of multiple antioxidant preparations.

Objective The intention of this study was to investigate the effect of selenium on pancreatic and remote organ injury in a well-validated experimental model of acute pancreatitis.

Methods Male Sprague-Dawley rats were randomly allocated to one of 3 groups (n=5/group) and sacrificed at 72 hours. Acute pancreatitis was induced by 250 mg per 100 g body weight of 20% L-arginine hydrochloride in 0.15 mol/L sodium chloride. Group allocations were: Group 1, control; Group 2, acute pancreatitis; Group 3, selenium.

Main outcome measures Serum amylase, anti-oxidant levels, bronchoalveolar lavage protein, lung myeloperoxidase activity, and histological assessment of pancreatic injury.

Results L-arginine induced acute pancreatitis characterised by oedema, neutrophil infiltration, acinar cell degranulation and elevated serum amylase. Selenium treatment was associated with reduced pancreatic oedema and inflammatory cell infiltration. Acinar degranulation and dilatation were completely absent. A reduction in bronchoalveolar lavage protein content was also demonstrated.

Conclusion Intravenous selenium given 24 hours after induction of experimental acute pancreatitis was associated with a reduction in the histological stigmata of pancreatic injury and a dramatic reduction in broncho-alveolar lavage protein content. Serum selenium fell during the course of experimental acute pancreatitis and this effect was not reversed by exogenous selenium supplementation.

INTRODUCTION

Oxidative stress, mediated by short-lived intracellular oxygen free-radical species is one of the mediators of acinar cell and remote...
organ injury in experimental acute pancreatitis (AP) [1, 2]. Corroborative evidence for the involvement of oxidative stress mediators is derived from studies demonstrating upregulation of the oxidative stress response genes c-fos, heme oxygenase and metallothionein during experimental acute pancreatitis [3]. These genetic changes are paralleled in the clinical state by depletion of serum anti-oxidants during acute pancreatitis with the degree of depletion corresponding to disease severity [4]. In intraperitoneal L-arginine-induced experimental acute pancreatitis we have previously demonstrated that exogenous anti-oxidant supplementation using a combination of n-acetylcysteine, selenium and vitamin C reduces stigmata of pancreatic injury when the compounds are administered early in the disease course [5]. However, a case-control study from this unit demonstrated no benefit from clinical administration of these multi-compound anti-oxidants in clinical acute pancreatitis although critically, many patients in the clinical study received anti-oxidants relatively late in the disease course [6]. In contrast, Angstwurm’s randomised clinical trial of administration of intravenous selenium to patients admitted to an intensive care unit with the systemic inflammatory response syndrome (SIRS) with intervention commencing on the day of admission revealed that treatment with selenium resulted both in a normalization of selenium levels and a reduction in organ failure [7].

Selenium is a co-factor for the antioxidant enzyme glutathione peroxidase (GPx) [8]. Glutathione peroxidase catalyses the reduction of both hydrogen peroxide and lipid hydroperoxides (GSH) [9] and as such acts as an intracellular defence against free radical injury [10].

The aim of the present study is to evaluate the effect of selenium in a well-validated experimental model of acute pancreatitis in which there is reliable acinar cell injury associated with a state of oxidative stress [11, 12] together with consistent extra-pancreatic end-organ effects [13, 14]. In particular, pulmonary oedema and neutrophil infiltrate are characteristic and replicate the common human disease variants.

**METHODS**

**Animal Care**

Fifteen adult male Sprague-Dawley rats with a median weight of 360 g (range: 270-420 g) were used in this study. There was no significant difference in animal weights between study groups (Kruskal-Wallis test: P=0.494). Standard diet and water were provided *ad libitum* throughout the study period.

**Model of Acute Pancreatitis and Study Design**

Acute pancreatitis was induced by intraperitoneal injection of L-arginine as previously described [13]. Two hundred and fifty milligrams per 100 g body weight of 20% L-arginine hydrochloride in 0.15 mol/L sterile sodium chloride solution was given at a time point designated 0 hours. The animals were sacrificed 72 hours after induction of experimental acute pancreatitis (or commencement of study in controls). Samples were collected for biochemical and histological assessment immediately after animal sacrifice. The animals were randomly allocated into 3 groups (n=5 per group) as follows.

**Group 1: Control.** No intervention was undertaken in these animals until sacrifice at 72 hours.

**Group 2: Acute Pancreatitis (AP).** Acute pancreatitis was induced at onset of experiment (0 hours) as described above. Intravenous (tail vein) bolus injections of (0.9%) sterile normal saline were given at 24 and 48 hours following induction of acute pancreatitis.

**Group 3: Selenium.** Intravenous tail vein bolus injections of 15μg/kg of selenium at 24 and 48 hours following induction of
experimental acute pancreatitis by intraperitoneal injection of L-arginine at 0 hours. The selenium used was in the form of sodium selenite and the concentration was derived from the protocol in clinical use in this institution at the time of the study which was a loading dose of 1,000 µg in a 70 kg adult at commencement of clinical treatment to be repeated 24 hours later [6].

The total injected volume was 5 mL per kg body weight for each group in order to conform to accepted practice [15] and to maintain consistency of resuscitative fluid volume between groups. The control and acute pancreatitis groups were run in parallel with a concurrent study and data from these two groups have been reported elsewhere [5, 16].

Biochemical Assessment

Serum was analysed for selenium, and the molar ratio of the 9-cis, 11-trans isomer of linoleic acid was expressed as a molar ratio of the parent 9,12 linoleic acid in serum. In brief, serum selenium was analysed by graphite furnace atomic absorption spectroscopy [17]. The lower limit of detection in our laboratory was 2.0 µg/L with a within batch coefficient of variation (CV) of 3.0% and a between batch CV of 5.0% [18]. Analysis of serum 9,12 linoleic acid and its 9,11 isomer was based on enzymatic hydrolysis, solid-phase sample preparation and high-performance liquid chromatography [19]. The lower limit of detection for linoleic acid was 20 µmol/L (within batch CV, 3.8%; between batch CV, 11.2%) and for 9,11 the lower limit for detection was 3.0 µmol/L (within batch CV, 4.3%; between batch CV, 7.0%). Results are expressed as molar ratio of 9,11 to 9,12 linoleic acid. Serum was also analysed for amylase content and albumin as a surrogate marker for haemoconcentration by the clinical biochemistry laboratory at Cork University Hospital.

Assessment of Pulmonary Injury

Pulmonary oedema and endothelial injury were assessed by analysis of bronchoalveolar lavage (BAL) fluid protein content [20]. Lung tissue myeloperoxidase concentration (MPO) (expressed as units of activity per g lung tissue) was assessed as an index of pulmonary neutrophil infiltration [21]. The value of measuring MPO activity to assess polymorphonuclear (PMN) infiltration has been previously reported [22].

Histological Assessment

Pulmonary and pancreatic parenchymal samples were buffered in standard 10% formalin prior to staining with haematoxylin and eosin. Qualitative examination of histologic samples was undertaken by a Consultant Histopathologist blind to specimen group allocation. A histological injury score was applied, derived from that utilised by Rakonczay et al. [23], by grading from 0 to 3 for the following components: oedema; polymorphonuclear (PMN) lymphocyte infiltration; mononuclear cell infiltration; acinar cell degranulation; and acinar cell dilation.

ETHICS

The study was conducted following approval from the Biological Services Unit at University College, Cork, Ireland. The animals received humane care according to the criteria outlined in the ‘Guide for the Care and Use of Laboratory Animals (1996)’ prepared by the National Academy of Sciences.

STATISTICS

Data are expressed as median (range) unless otherwise stated. Statistical analysis was undertaken by unpaired, two-tailed non-parametric test (Mann-Whitney U-test) unless otherwise stated. Kruskal-Wallis was also used. Statistical significance was accepted at the P<0.05 levels. The GraphPad Instat statistics package (GraphPad Software, San Diego, California) was used.

RESULTS

Biochemical Analysis (Table 1)

Serum Amylase. The serum amylase was elevated (P=0.008) in animals with AP when compared to control. The serum amylase in
the selenium group was similar to that in animals in the AP group.

**Serum Selenium.** The serum selenium was lower ($P=0.016$) in the AP group when compared to controls. The serum selenium levels were also lower in animals given selenium ($P=0.008$) when compared to the control group.

**Serum 9,11/9,12 Linoleic Acid Ratio.** Serum 9,11 linoleic acid concentrations were below the level of detection in all study groups.

**Serum Albumin.** There was no significant difference in serum albumin concentrations between groups.

**Pulmonary Injury (Table 2)**

**Myeloperoxidase Activity (MPO) (Figure 1)** MPO activity was greater ($P=0.008$) in the AP group compared to control. MPO activity was found to be similar in the selenium group compared to animals with AP and elevated in comparison to control animals ($P=0.008$ vs. control).

**Bronchoalveolar Lavage (BAL) Protein Content (Figure 2).** The BAL protein content was elevated ($P=0.008$) in animals with acute pancreatitis compared to control. The BAL protein content was reduced in animals receiving selenium ($P=0.008$) compared to the AP group.

**Histological Assessment (Table 3)**

**Pancreatic Injury.** The pancreatic injury in this model was characterised by oedema,

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**Table 1. Biochemical analysis: median (range).**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 2 (AP)</th>
<th>Group 3 (Selenium)</th>
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<tbody>
<tr>
<td>Amylase (U/L)</td>
<td>4.950 (4,210-5,100)</td>
<td>7.446 (6,537-9,787)</td>
<td>6.834 (4,948-9,845)</td>
</tr>
<tr>
<td>Selenium (µg/L)</td>
<td>505 (465-564)</td>
<td>460 (432-469)</td>
<td>358 (277-396)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36 (36-39)</td>
<td>35 (34-36)</td>
<td>32 (31-35)</td>
</tr>
</tbody>
</table>

**AP:** acute pancreatitis  
$^a$ $P$ vs. Group 1 (Control)  
$^b$ $P$ vs. Group 2 (Acute pancreatitis)

**Table 2. Pulmonary injury: median (range).**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 2 (AP)</th>
<th>Group 3 (Selenium)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO (U/g)</td>
<td>0.64 (0.41-0.99)</td>
<td>2.33 (2.31-2.78)</td>
<td>2.82 (1.72-3.04)</td>
</tr>
<tr>
<td></td>
<td>$P=0.008^a$</td>
<td>$P=0.008^a$</td>
<td>$P=0.151^b$</td>
</tr>
<tr>
<td>BAL protein (µg/mL)</td>
<td>125 (82-147)</td>
<td>2,217 (1,192-2,809)</td>
<td>327 (209-731)</td>
</tr>
<tr>
<td></td>
<td>$P=0.008^a$</td>
<td>$P=0.008^a$</td>
<td>$P=0.008^b$</td>
</tr>
</tbody>
</table>

**AP:** acute pancreatitis  
MPO: myeloperoxidase activity  
BAL: bronchoalveolar lavage  
$^a$ $P$ vs. Group 1 (Control)  
$^b$ $P$ vs. Group 2 (Acute pancreatitis)
inflammatory cell infiltration (both neutrophil polymorphonuclear leucocytes and mononuclear cells); acinar cell degranulation and dilatation. Acinar cell necrosis was not seen and the architecture of the islets of Langerhans and the pancreatic ducts was preserved (Figure 3). Selenium supplementation was associated with marked reduction in histological evidence of pancreatic injury. In the selenium group pancreatic oedema was reduced compared to those animals with AP; inflammatory cell infiltration (both PMNs and monocytes) was absent and there was no evidence of acinar cell degranulation or dilation on light microscopy (Figure 4).

DISCUSSION

Depletion of plasma selenium is well documented in critical illness and may relate to cellular oxidative stress [24, 25]. Although it is not established whether this selenium depletion is a central component of critical illness or an epiphenomenon, clinical studies in critical illness have demonstrated beneficial outcomes from selenium supplementation [7,26]. The present study addresses the question of whether selenium supplementation after induction of acute pancreatitis normalises stigmata of pancreatic injury. In this 72-hour model, the concentration of L-arginine used produces a moderate oedematous pancreatitis with hyperamylasaemia but without hypoalbuminaemia. In this study, serum selenium was lower in experimental acute pancreatitis than in controls. Selenium supplementation did not prevent this phenomenon and selenium levels were reduced in the group receiving selenium supplementation. The concentration of 15 µg/kg selenium was based on direct extrapolation from the doses of selenium used in clinical studies in acute pancreatitis in this unit [6] but it is possible that higher concentrations may have produced different effects on serum selenium levels.

Table 3. Histological injury score expressed as mean±SD.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 1 (AP)</th>
<th>Group 2 (Selenium)</th>
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</thead>
<tbody>
<tr>
<td>Oedema</td>
<td>0</td>
<td>1.4±0.2</td>
<td>0.2±0.4</td>
</tr>
<tr>
<td>PMN infiltration</td>
<td>0</td>
<td>1.4±0.4</td>
<td>0</td>
</tr>
<tr>
<td>Mono infiltration</td>
<td>0</td>
<td>1.2±0.3</td>
<td>0</td>
</tr>
<tr>
<td>Acinar degranulation</td>
<td>0</td>
<td>2.4±0.2</td>
<td>0</td>
</tr>
<tr>
<td>Acinar dilation</td>
<td>0</td>
<td>2.9±0.2</td>
<td>0</td>
</tr>
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PMN: polymorphonuclear cell
Mono: mononuclear cell

Figure 3. Pancreatic injury in L-arginine-induced experimental acute pancreatitis (H&E x40). Note the presence of oedema, inflammatory cell infiltration, acinar cell degranulation, and dilatation.

Figure 4. H&E preparation of pancreatic parenchyma from selenium treated animal (x40). Note the relatively normal acinar architecture.
significant reduction in bronchoalveolar lavage protein content. Selenium modified histological features of pancreatic injury and abolished PMN and mononuclear cell infiltration and features of acinar degranulation and dilatation. These features were striking and consistently reproduced, however the limitations of histologic injury scores must be acknowledged in that histologic injury is a continuum and not a categorical variable and prone to sampling bias.

Lipid peroxidation is a common feature of oxidative stress injury and the ratio of 9,11/9,12 linoleic acid has been shown to be an index of cellular injury in clinical acute pancreatitis (2). However quantifiable levels of 9,11 linoleic were not detected in this experimental model suggesting an alternative pathway for lipid peroxidation in rats. Consequently the 9,11/9,12 linoleic acid ratio is not a useful index for oxidative stress injury in this model of acute pancreatitis.

In summary, this study demonstrates that intravenous selenium given 24 hours after induction of experimental acute pancreatitis was associated with a reduction in the histological stigmata of pancreatic injury and a dramatic reduction in broncho-alveolar lavage protein content. Serum selenium fell during the course of experimental acute pancreatitis and this effect was not reversed by exogenous selenium supplementation. The question of whether anti-oxidant therapy is beneficial in pancreatitis remains unanswered to date. This small experimental study provides novel insight in that in a well-validated model, intervention with selenium was commenced 24 hours after induction of experimental acute pancreatitis and was associated with amelioration of pancreatic injury and lung injury. The findings of this study suggest that intervention with selenium is worthy of more detailed experimental and clinical evaluation in acute pancreatitis.

Keywords Antioxidants; Pancreatitis; Selenium; Therapeutics

Abbreviations AP: acute pancreatitis; BAL: bronchoalveolar lavage; CV: coefficient of variation; MPO: myeloperoxidase activity

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