Antioxidant potential of i.v. fluids

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Summary

In acute clinical settings where there may be a role for antioxidant therapy, patients receive large amounts of i.v. fluids which may have antioxidant activity. To investigate such effects the antioxidant capacity of nine i.v. fluids was measured (Gelofusine, Haemaccel, 20% mannitol, 4.5% human albumin solution, fresh frozen plasma, aprotinin, N-acetylcysteine and two hydroxyethyl starch solutions). Results are expressed as mean mmol litre\(^{-1}\) Trolox equivalents. Mannitol 20% and the hydroxyethyl starch solutions had no antioxidant activity. Protein-containing solutions (gelatins, albumin and aprotinin) had antioxidant activity 50–66% that of plasma: Gelofusine 0.85 mmol litre\(^{-1}\); Haemaccel 0.78 mmol litre\(^{-1}\); 4.5% albumin 0.95 mmol litre\(^{-1}\); aprotinin 0.80 mmol litre\(^{-1}\); fresh frozen plasma 1.45 mmol litre\(^{-1}\). However, none was nearly as potent an antioxidant as the clinical preparation of N-acetylcysteine, with an antioxidant activity of 502 mmol litre\(^{-1}\). Studies of antioxidant therapy may need to take account of the antioxidant effect of i.v. fluids. (Br. J. Anaesth. 1997; 78: 757–759).

Key words

Fluids, i.v.

Methods and results

The antioxidant capacity of nine fluids was measured using the method described by Miller and colleagues.\(^4\) In brief, this assay relies on conversion of a phenothiazine 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to a long-lived radical cation (ABTS\(^{•+}\)) by hydrogen peroxide in the presence of metmyoglobin acting as a peroxidase. ABTS\(^{•+}\) was produced in a linear manner and the concentration measured by spectrophotometry 10 min after initiation of the reaction. Addition of antioxidants to the assay delays the production of ABTS\(^{•+}\) so that there is apparent inhibition of oxidation compared with saline controls. A standard curve was constructed using the water-soluble vitamin E analogue Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). All reagents were obtained from Sigma (UK), except Trolox which was obtained from Aldrich (UK). Metmyoglobin was purified as described by Miller and colleagues to give a final purity of >90% total haem protein.

A standard curve was plotted as percentage inhibition of ABTS\(^{•+}\) formation when Trolox 0.5, 1.0, 1.5, 2.0 and 2.5 mmol litre\(^{-1}\) was present compared with controls without Trolox. Ten measurements were made for each point and the mean was used to plot the standard curve. There was an sd of 7% about each point indicating a precision similar to that in the original description of the method.\(^4\)

The total antioxidant capacity of any fluid (or dilution of it) added in place of Trolox to the assay can be expressed as mmol litre\(^{-1}\) Trolox equivalent activity. The fluids studied were Gelofusine (Braun), Haemaccel (Hoerst), 20% mannitol (Baxter), 4.5% human albumin solution (Bio Products Laboratory), fresh frozen plasma (National Blood Transfusion Service), aprotinin (Bayer), N-acetylcysteine (Evans) and two 6% hydroxyethyl starch solutions (Dupont and Laevozan).

The antioxidant capacity of each fluid was measured neat except for N-acetylcysteine (formulation concentration 200 mg ml\(^{-1}\)). This was diluted

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to the recommended initial therapeutic concentration of 150 mg kg⁻¹ in 200 ml for a 70-kg patient as this is the fluid normally infused. In fact, this solution was so potent that it had to be diluted further 200-fold with phosphate-buffered saline to fall within the Trolox reference range.

The antioxidant capacity of each fluid was measured 10 times and the results, which appeared to have a normal distribution, expressed as mean (SD). The statistical package used was StatView 4.1 Power PC version for Apple Macintosh computers (Abacus Concepts, Inc).

The results of these experiments are shown in Table 1. All protein-containing fluids (gelatins, 4.5% albumin, plasma and aprotinin) had antioxidant activity. The hydroxyethyl starch and mannitol solutions had no antioxidant activity. As would be expected, N-acetylcysteine had potent antioxidant activity in this system. The 200-fold dilution had an antioxidant capacity of 2.51 (0.07) mmol litre⁻¹, an equivalent of 502 mmol litre⁻¹ for the solution used clinically. When the relative concentrations are accounted for, N-acetylcysteine 1 mmol litre⁻¹ had antioxidant activity equivalent to Trolox 1.56 mmol litre⁻¹.

### Table 1. Mean (SD) antioxidant capacity of nine i.v. fluids

<table>
<thead>
<tr>
<th>Fluid</th>
<th>Mean Trolox equivalent (mmol/litre⁻¹)</th>
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</thead>
<tbody>
<tr>
<td>Gelofusine</td>
<td>0.85 (0.12)</td>
</tr>
<tr>
<td>Haemaccel</td>
<td>0.78 (0.14)</td>
</tr>
<tr>
<td>20% Mannitol</td>
<td>0.0 (0.19)</td>
</tr>
<tr>
<td>4.5% Human albumin</td>
<td>0.95 (0.08)</td>
</tr>
<tr>
<td>Fresh frozen plasma</td>
<td>1.45 (0.10)</td>
</tr>
<tr>
<td>Hespan—Dupont</td>
<td>0 (0.21)</td>
</tr>
<tr>
<td>Hespan—Laevosan</td>
<td>0 (0.22)</td>
</tr>
<tr>
<td>Aprotinin—Trasylol</td>
<td>0.80 (0.07)</td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>502 (14)</td>
</tr>
</tbody>
</table>

Comment

The finding that all the protein solutions have antioxidant activity is to be expected. This is because proteins have amino acid constituents with antioxidant activity such as sulphhydril, hydroxyl and carboxyl groups. In the article by Miller and colleagues describing the method used here, evidence was presented that albumin is the largest single contributor to total plasma antioxidant capacity. It has an antioxidant potency equivalent to Trolox and a high plasma concentration compared with other antioxidants, which make up the difference between the measured albumin and total plasma antioxidant capacities. In this study, measurements of total plasma antioxidant capacity, represented by fresh frozen plasma, of 1.45 mmol litre⁻¹ agreed with those of Miller and colleagues whose mean value was 1.46 mmol litre⁻¹ with a similar degree of variation. This suggests a comparable performance of this assay in our laboratory to the original report.

It is perhaps surprising that the gelatin solutions, Gelofusine and Haemaccel, were nearly as potent as the 4.5% albumin solution. Gelatin consists largely of polyglycine which, because it has only the simplest amino acid side chain (–H) would not be expected to have antioxidant activity. It may be that gelatin modification by succinyly- (Gelofusine) or urea- (Haemaccel) linkage might increase the antioxidant activity. Both urea and succinate, a dicarboxylate, are antioxidants. The similar antioxidant potentials of the gelatin solutions and 4.5% albumin cannot be explained in terms of a higher protein concentration in the gelatin solutions as Gelofusine is a 4% solution and Haemaccel a 3.5% solution.

In common with the gelatin solutions, the clinical formulation of aprotinin has an antioxidant potential which approaches that of 4.5% albumin solution. This may be an additional attraction when used for its antifibrinolytic action during cardiopulmonary bypass.

The rinding that the hydroxyethyl starch solutions had no antioxidant activity was not surprising, as starches are relatively inert compared with proteins. Starches do have hydroxyl (–OH) groups which can have antioxidant activity. However, in polyhydroxy compounds, such as starch, they are much less reactive than in monohydroxy compounds which include some potent antioxidants.

Mannitol had no activity in either our experiments or those of Miller and colleagues. In many articles mannitol is claimed to be a potent hydroxyl radical scavenger. If this is so, then it may not be an antioxidant in this particular system where the following reactions are thought to occur:

\[
\text{HX-MbFe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{X-Mb}^{3+} + \text{H}^+ + \text{H}_2\text{O}
\]

Hydrogen peroxide (H₂O₂) oxidizes metmyoglobin (HX-MbFe³⁺⁺) to form ferrylmyoglobin (X-Mb³⁺⁺-OH), a highly reactive bi-radical, which oxidizes ABTS to its stable radical cation (ABTS⁺) with regeneration of metmyoglobin. No hydroxyl radicals are formed in this system and therefore a pure hydroxyl radical scavenger would not inhibit these reactions. However, in experiments such as those of Del Maestro and colleagues which are cited widely by authors referring to mannitol as a potent hydroxyl radical scavenger, a mixture of reactive oxygen species was present. Therefore, it is not clear that mannitol is a pure hydroxyl scavenger. There are two explanations for what was observed: either mannitol is not an effective antioxidant (indeed some work from the organic chemistry literature suggests that polyhydroxy compounds such as mannitol would not be potent antioxidants) or that mannitol scavenges reactive oxygen species, but the radical produced is itself highly reactive and contributes to oxidant stress. Whatever the explanation, this must cast doubt on the potential usefulness of mannitol as a therapeutic antioxidant.

As expected, the most potent antioxidant solution was N-acetylcysteine which is probably the best available agent to investigate for any therapeutic role. However, being water soluble, it may not be the best agent to protect lipid membranes which are highly susceptible to oxidant-induced injury.

In summary, except for N-acetylcysteine, all i.v. fluids investigated had no or only modest antioxidant activity. They are therefore probably of little therapeutic use. However, in clinical studies describing a small antioxidant effect of any therapy,
investigators may need to be aware of a possible confounding effect of protein-containing i.v. fluids.

**Acknowledgements**

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**References**