Pretreatment with Antioxidants Decreases Damage Due to Reperfusion of Isolated Intestines in the Absence of Leucocytes

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Summary

The effect of antioxidants in an experimental model of intestinal reperfusion "in vitro" is studied. The perfusion is carried out in the absence of leucocytes in order to analyze the direct role of oxygen free radicals in the reperfusion damage and the contribution of antioxidants to block their deleterious effect. Enzymatic movements are completely reverted by antioxidants (superoxide dismutase, folinic acid and alpha tocopherol), and electrolytes and pH alterations are partially improved.

Introduction

In our previous studies we have found that some antioxidant drugs (superoxide dismutase and folinic acid) decrease both the local damage to the intestine and the mortality rate following one or two hours of absolute interruption of blood flow to the small intestine[1]. These two drugs, and also alpha-tocopherol, have improved all hemodynamic parameters following sixty minutes of intestinal ischemia[2]. However, it could be that these good effects were consequence of a mechanism of action different from antioxidation. In fact, folinic acid has proved to be a potent hepatotrophic agent[3].

It is well known that oxygen free radicals generated during the early phase of reperfusion induce activation of leucocytes, which attack not only the endothelial cells of the gut, but any capillaries throughout the hole organism. The clinical translation is a multiorganic failure which leads to death of the animal. But it has also been accepted that oxygen free radicals directly

affect the intestinal mucosa, and that antioxidant drugs block this effect. The question that arises is whether antioxidants have any direct effect on the leucocyte-induced damage or not.

Leucocytes are said to be the agent mainly responsible for intestinal reperfusion damage. In the "in vitro without blood cells" model we have been testing for the last two years, we checked the scarce benefit of antioxidant drugs in reversing the damage due to ischemia. Now we wanted to investigate whether the same antioxidants (AO) are efficient as damage blockers when used before and after the ischemic period.

Materials and Methods

Male Wag rats have been used. The superior mesenteric artery and the portal vein were canulated, and both the jejunum and ileum were excised, cleansed with heparinized Ringer solution, and perfused with 10 ml of either Ringer (groups 1 and 2) or Ringer supplemented with AO (folinic acid 0.21 ml/ml, alfatocopherol 1.6 ml/ml and SOD 0.58 ml/ml). The organ was placed in a bath at 40°C for 60 minutes, and then it was perfused for 30 minutes with our acellular reperfusion solution (ARS) (group 1) or ARS+antioxidants (groups 2 and 3; Folinic acid 0.37 mg/ml, Superoxide dismutase 6.2 I.U./ml, Alpha tocopherol acetate 1.7 mg/ml). The perfusate contains the same concentration of electrolytes as the normal blood, plus glucose, some vasoactive agents and oxygen[4]. Our system provides a pulsatil flow quite similar to the normal blood flow and was kept at a constant rate of 7.9 ml/min.

Samples from the perfusate and portal drainage were collected at 0, 5, 15 and 30 minutes of perfusion, and electrolytes, pH, glucose, CPK, LDH and PA were determined. Once the perfusion was completed, six consecutive fragments of distal ileum (1 cm long) were obtained and embedded in paraffin. Statistical analysis (T test) have been performed with Prisma ®.

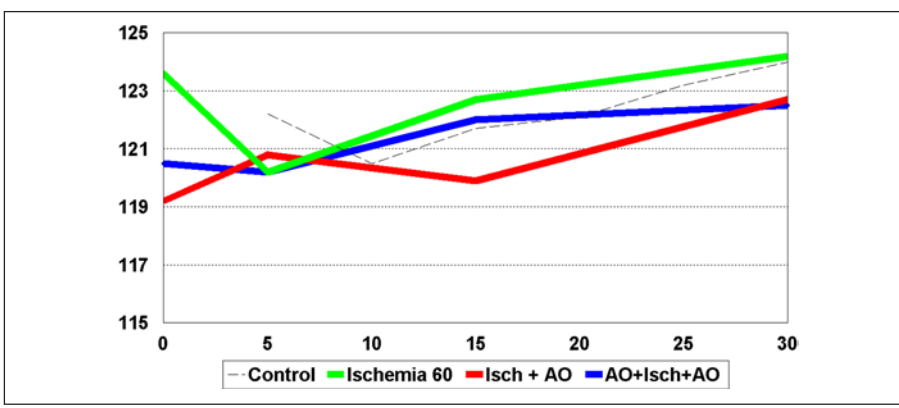

Figure 1. Sodium concentrations in the portal effluent of intestines perfused for thirty minutes.

Results

The initial hyponatremia is progressively reduced, approaching normal values after thirty minutes of perfusion. Adding antioxidants has not modified this behavior. No significant differences between the four groups may be observed (figure 1).

If we focus now on calcium concentrations (figure 2), we may observe in the upper curve that the control organs show normal values through out the whole period of perfusion. On the other hand, looking at the organs perfused after sixty minutes of normothermic ischemia, we see how the curve starts with a decreased concentration of Calcium which is not completely normalized at the end of the experiment. In the third curve, we have represented the data from AO-treated intestines. Now, it looks very like the previous one at the beginning, but it shows a steady improvement, reaching fully normal values after 15 minutes. The results are even better when AO were given prior to ischemia.

We have found a different pattern when analyzing the potassium (figure 3). The control organs do not recover from the initial hyperpotasemia until the very end of the experiment, while those perfused after one hour of ischemia had nearly normal values within fifteen minutes after starting the

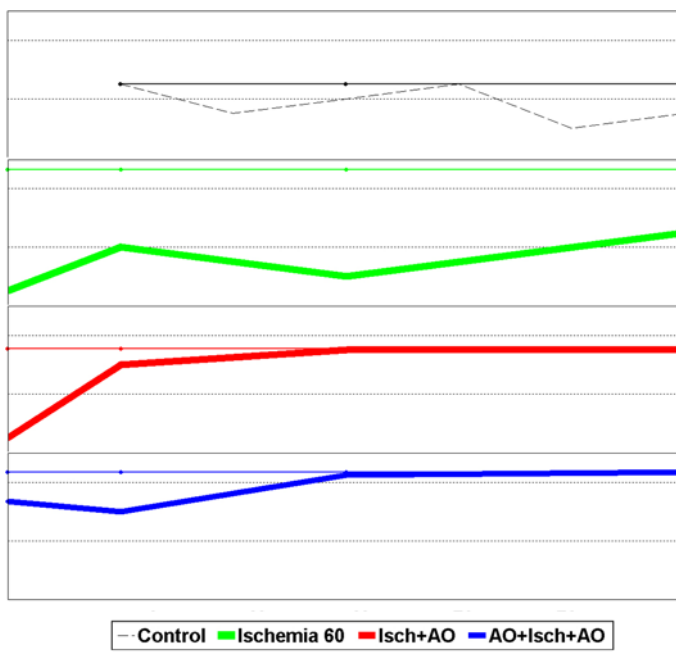

Figure 2. Calcium concentrations in the portal effluent of intestines perfused for thirty minutes. To make clearer the differences each graph contains a thin line marking calcium concentrations in the perfusate (prior to circulating through the intestine).

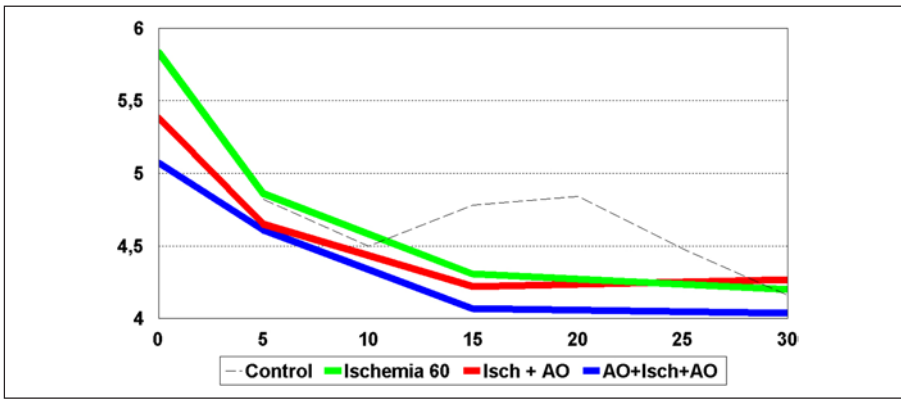

Figure 3. Potassium concentrations in the portal effluent of intestines perfused for thirty minutes.

perfusion. We have also found a slightly better result when adding antioxidants to the perfusate and even more when pretreating them with AO: if we look at the data shown in figure 3, the values in the series including antioxidants are always lower than the non treated series, though these differences are not statistically significant.

Something similar happens to the values of pH (figure 4). In the control organs we start with normal values, which progressively decrease, while the ischemic intestines recover through the perfusion period, not finding differences when adding antioxidants to the perfusate. However, in the case of the intestines pretreated with AO the initial decrease is not so significant, and a normal pH is reached in the 15th minute.

The oxygen extraction somehow assesses the metabolic activity of the

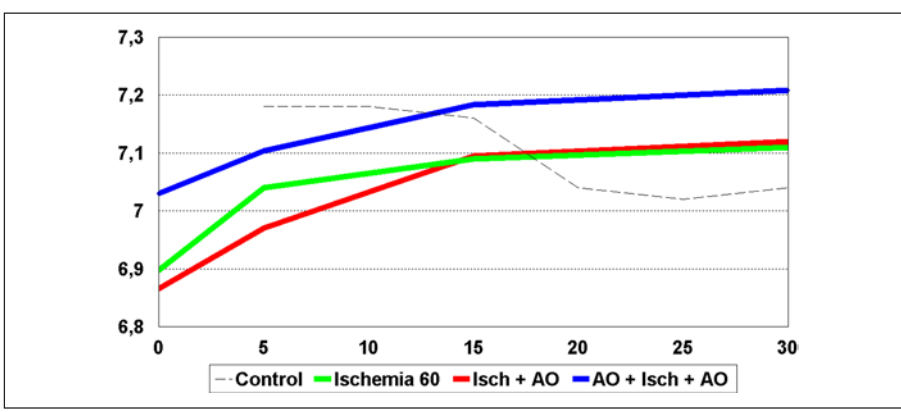

Figure 4. The lines represent pH values in the portal effluent of intestines perfused for thirty minutes. The pH of the perfusate (as measured by the automatic analyzer) was 7.2 (equivalent to 7.44 measured by a conventional pHmeter).

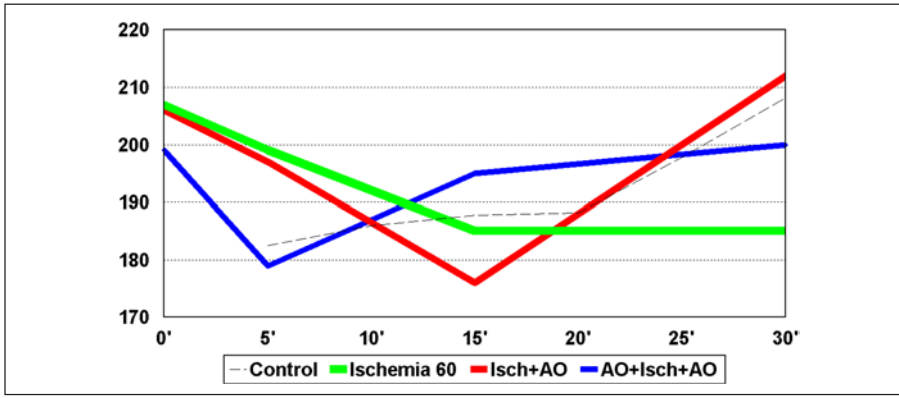

Figure 5. Oxygen extraction represents the difference between the partial pressure of oxygen in the perfusate and in the portal effluent. Values on the graph represent mm Hg.

organ. In the control organs, after fifteen minutes of perfusion, oxygen consumption shows a steady increase (figure 5). On the other hand, ischemic intestines show high initial values followed by a drop from which they do not recover. When AO were added to the perfusate, the curve initially paralleled the non-treated one, but from the 15th minute there was a significant recovery. And last of all, in these intestines pretreated with AO the initial drop was more abrupt, but it was followed by an significant recovery.

In the ischemic intestines, CPK and LDH remained within the range of normal values; only Alkaline Phosphatase was elevated during the first minutes, reaching non-detectable levels after 15 minutes of perfusion.

This mild elevation, was blocked when adding AO to the perfusate (figure 6). Thus, tissue damage (assessed by enzyme movements) in this model

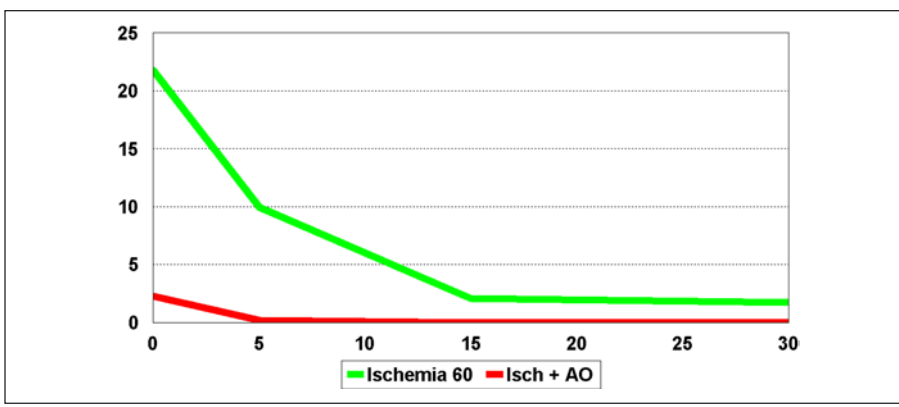

Figure 6. Alkaline phosphatase. The values from the control and the pretreated series are omitted as they are always below 0.2.

is very small, and completely reverted by AO.

Conclusions

Enzymatic studies suggest that the damage induced by reperfusion in isolated intestines is small, with only scarce amounts of alkaline phosphatase detectable in the portal effluent. However, pathological studies of the intestinal mucosa are necessary to confirm this observation.

The alterations observed in oxygen consumption and Ca, K and pH levels, are slightly corrected by the addition of antioxidants to the perfusate; but a more vigorous effect is obtained if these drugs are given also previous to the ischemic aggression. These data suggest that the effect of antioxidants is –at least- partially independent on the leucocyte pathway.

References

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