Glycine reduces liver lipid peroxidation in neonatal hypoxia/reoxygenation-induced necrotizing enterocolitis

A glicina reduz a peroxidação lipídica hepática na enterocolite necrotizante neonatal induzida pela hipóxia-reoxigenação

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ABSTRACT

Objective: To assess the protective effect of glycine in liver of neonatal hypoxia/reoxygenation-induced necrotizing enterocolitis in rats. Methods: Forty-four (44) neonatal Wistar rats were distributed into three groups: G1 – normal control group (n=12); G2 Group (n=16) with animals that underwent hypoxia-reoxygenation; G3 Group (n=17) with animals submitted to hypoxia-reoxygenation following a 5% intraperitoneal glycine infusion. The groups were subdivided into A: euthanasia 12 h after hypoxia-reoxygenation and B: euthanasia 72 h after hypoxia-reoxygenation. The liver was removed for determination of tissue malondialdehyde. Results: Malondialdehyde values did not differ significantly in subgroups G1 and G3. The animals in G2A had mean malondialdehyde values significantly lower than those in G2B. Malondialdehyde values did not differ significantly for animals in subgroup A. In subgroup B, the malondialdehyde values did not differ significantly among the animals in G1 and G3. G2 animals had mean malondialdehyde values significantly higher than G3 animals. Conclusion: Glycine reduces liver lipid peroxidation in hypoxia/reoxygenation-induced necrotizing enterocolitis.

Keywords: Glycine; Enterocolitis, necrotizing; Ischemia; Liver/ injuries

INTRODUCTION

Despite significant advances in care provided to high-risk newborns, necrotizing enterocolitis (NEC) continues to be the most important cause of mortality and morbidity in low-birth-weight infants. NEC is the most common gastrointestinal emergency in neonatal
OBJECTIVE
To assess the capacity of glycine to prevent liver lipid peroxidation caused by hypoxia-reoxygenation in a previously described experimental model of NEC(10) was assessed.

METHODS
Experimental Design. The animal experiment was approved by the Research Bioethics Committee of the Universidade Federal de São Paulo - UNIFESP-EPM, and registered under number 0560/04.

Forty-four (44) neonatal OUT B EPM-1 Wistar rats (Rattus norvegicus albinus, Rodentia mammalia), from a litter of six female rats and weighing 4 to 6 grams, were used. The animals were randomized into three groups: normal control G1 group (n = 12); G2 group (n = 15) with animals undergoing HR; G3 group (n = 17) with animals undergoing HR following a glycine intraperitoneal infusion. The animals underwent hypoxia in a rodent CO₂ kill chamber where they received an air flow containing 100% CO₂ for 5 minutes. Following hypoxia the animals were resuscitated with an air flow containing 100% O₂ for 5 minutes and then kept close to their respective mothers in a normothermic environment(9). All animals were given breast milk before and after the procedure. In Group 3, the animals received 0.2 ml of 5% glycine solution in saline solution(12-14). The glycine injection was administered 30 minutes prior to hypoxia-reoxygenation and it was maintained once a day until animal euthanasia. The animals were submitted to euthanasia by decapitation. The groups were subdivided to demonstrate that liver injury was not caused by HR alone. In subgroup A, euthanasia occurred 12 hours after HR and in subgroup B, 72 hours after HR.

The liver was removed in a block and immediately frozen at -80°C for subsequent homogenization and measurement of tissue MDA.

Determination of MDA. MDA is a final product of lipid peroxidation and a well-established parameter to determine the increase of free radicals in intestinal tissue(6). To determine MDA levels, the thiobarbituric acid (TBA) reaction proposed by Kohn and Liversedge(15) was used and the levels were expressed in nmol MDA/mg of protein. The protein content of the homogenate was determined by the coomassie brilliant blue (CBB) procedure. Tissue samples were defrosted, weighed, and a volume equivalent to five times the weight of TRIS 0.01M/pH 7.4 buffer solution was then added. Tissue samples were homogenized in ice bath four times, for 30 seconds each, and subsequently centrifuged for 5 minutes at 10000 rpm, at 4°C.
**Results**

MDA levels of the groups are shown in table 1. MDA values did not differ significantly among the subgroups in group G1 (p = 0.901) and group G3 (p = 0.094). The animals in group G2 subgroup A had mean MDA values significantly lower than those in subgroup B (p = 0.020).

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Mean (nmol MDA/mg protein)</th>
<th>SE</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>A</td>
<td>0.8310</td>
<td>0.1848</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.8043</td>
<td>0.0946</td>
<td>6</td>
</tr>
<tr>
<td>G2</td>
<td>A</td>
<td>0.7508</td>
<td>0.1135</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.9328</td>
<td>0.3924</td>
<td>8</td>
</tr>
<tr>
<td>G3</td>
<td>A</td>
<td>1.0662</td>
<td>0.1178</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.6891</td>
<td>0.1552</td>
<td>10</td>
</tr>
</tbody>
</table>

MDA values did not differ significantly in subgroup A (p = 0.256). In subgroup B, MDA values did not differ significantly in groups G1 and G3 (p = 0.949). The animals in group G2 had mean MDA values significantly higher than those in G1 (p = 0.023). Group G2 had mean MDA values significantly higher than G3 (p = .004).

**Discussion**

NEC is the most frequent and lethal disease affecting the gastrointestinal tract of preterm infants. Although the etiology of NEC has not been well defined yet, hypoxia definitely plays an important role in its pathogenesis\(^{(4-9)}\). Several animal models were proposed to show the relevance of hypoxia in the development of NEC-associated lesions\(^{(4-9)}\).

One of the reasons accepted to explain hypoxia-associated lesions is that neonatal asphyxia would lead to a redistribution of blood flow by triggering splanchnic vasoconstriction, diverting the flow to vital organs such as the heart and brain and causing intestinal ischemia\(^{(5)}\). Several mechanisms are involved in the onset and progression of this ischemia-associated lesion, such as increased production of hyperreacting peroxides, increased synthesis of adhesion molecules with neutrophilic infiltration, increased lipid peroxidation and increased production of inflammatory mediators such as cytokines\(^{(17)}\).

Lipid peroxidation is a complex process that can occur in biological membranes composed of molecular oxygen-reactant polysaturated fatty acids, leading to production of lipid hydroperoxides and their metabolites. Most cases involving lipid peroxidation start from a chain reaction that spreads out, mediated by the presence of free radicals. Lipid hydroperoxides accumulate in the membrane, inactivating its receptors...
and enzymes, affecting its functions, making it unstable and permeable to ions. A simple method of high sensitivity, very much used as a lipid peroxidation marker, involves thiobarbituric acid-reactive substances, such as lipid hydroperoxide derivatives. Hence, MDA is an adequate indicator of lipid peroxidation caused by free radicals(8).

Intestinal lesion in NEC causes damage to the affected organ, and, by triggering the release of inflammatory mediators into the blood stream, it can lead to dysfunction and failure of multiple organs, which is the most common cause of morbidity and mortality in necrotizing enterocolitis patients(2). The liver is positioned to first encounter these toxic mediators released from the intestine(5).

Glycine is a non-essential amino acid that protects the gut against lesions caused by the ischemia-reperfusion phenomenon(12-14). It is considered an anti-inflammatory and immune-modulating agent that has a direct cytoprotective function(10). Lee et al.(12), in a model of intestinal ischemia and reperfusion, showed that local 20% glycine mesenteric intravenous infusion increased mucosal protein and DNA content, reduced the intestinal myeloperoxidase activity and maintained increased mucosal protein and DNA content, reduced the intestinal myeloperoxidase activity and maintained glutaminase activity in the mucosa. Two other studies(13-14), also in an intestinal HR model in rats, showed the protective effect of glycine used in systemic intravenous infusion, by reducing the apoptosis cascade(13) and preserving the integrity and contractility of the intestinal wall(14). MDA intestinal levels, in our previous study using the NEC model proposed by Okur et al.(9), showed glycine as able to prevent lipid peroxidation. The group undergoing HR had mean MDA intestinal values significantly higher than those in the group undergoing HR and previously protected by using glycine (p = 0.021)(11).

Several techniques were used in an attempt to prevent multisystem organ failure due to intestinal ischemia-reperfusion injury. Vejchapipat et al.(15) showed that moderate hypothermia ameliorated liver energy failure after intestinal HR using magnetic resonance spectroscopy. Ferrer et al.(19) demonstrated that somatostatin and N-acetylcyesteine might improve prognosis and survival of patients with multiple organ failure mediated by oxidative stress after intestinal ischemia. Horie et al.(20) suggested that low-dose ethanol attenuates the gut ischemia-reperfusion hepatic microvascular dysfunction and sequential liver injury by increasing sinusoidal NO levels.

The group undergoing HR after 72 hours had mean MDA hepatic values significantly higher than those in the group undergoing HR and previously protected by the use of glycine (p = 0.004). The absence of difference between the control group and the group that used glycine (p = 0.949) showed that the level of protection provided by glycine in liver was so important that it provided a peroxidation level similar to that of normal control rats.

In the group submitted to HR after 12 hours, the MDA values did not differ significantly in the control group, suggesting that the liver peroxidation injury was not caused only by HR (the presence of intestinal lesion is required).

CONCLUSION

Our findings support the hypothesis that glycine reduces blood release of lipid peroxidation products from gut; thus, it prevents the increase of lipid peroxidation of liver.

It remains to be known, maybe in a not very distant future, to what extent such findings can actually benefit infants with NEC. Changing the history of this disease that still claims many lives among low-birth-weight infants is essential.

ACKNOWLEDGEMENTS

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REFERENCES