Oxidative stress and non-enzymatic antioxidant status in dogs with aspirin induced gastric mucosal injury

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SUMMARY

Eight (4 females and 4 males) adult and clinically healthy cross-bred dogs were used to evaluate plasma concentrations of malondialdehyde (MDA), glutathione (GSH) and vitamin C in order to establish their relevance in the pathological mechanism in aspirin-induced gastric mucosal injury in dogs. Dogs were given aspirin orally at a dose of 200 mg/kg body-weight for once after fasting for 12 hours. Endoscopic examinations of the upper gastrointestinal tract and biochemical analyses were performed before and 1, 4 and 7 days after aspirin administration. First day after aspirin administration, hemorrhagic, linear erosive lesions of the gastric mucosa could be observed in all dogs. Mean plasma MDA concentrations were significantly (p<0.01) increased in first day after aspirin administration while plasma vitamin C and GSH concentrations were decreased. In conclusion, our study shows that an increased lipid peroxidation may play an important role in the formation of mucosal injury induced by aspirin in dogs.

Keywords : Aspirin, gastric mucosal injury, oxidative stress, GSH, Vitamin C, dog.

Introduction

Peptic ulcer and gastritis have multi-etiopathogenetic factors. It is widely accepted that the pathogenesis of peptic ulcer is complex and still not completely understood. Increased acid secretion and pepsin activity, reduced mucus and bicarbonate secretion, enhanced contractility of the gastric wall and reduced gastric mucosal blood flow represent some of the establish pathogenic factors of gastric ulceration. Recent data point the attention on stressors of physical and chemical origin that can damage gastric mucosa through enhanced production of oxygen species and oxidative stress. This mechanism has been shown to be involved in the gastric ulceration caused by stress [5], ethanol [14, 26, 28], pyloric ligation [37], Helicobacter pylori [27] or ischemia / hypotensive shock [9].

Lipid peroxidation mediated by oxygen radicals is considered as an important cause of cell membrane damage and destruction, because a single initiating event can result in the conversion of hundreds of fatty acid side chains into lipid peroxides, altering the structural integrity and biochemical functions of the membrane [11, 23]. Plasma MDA is a complex marker of tissue lipid peroxidation [12]. Vitamin C -also referred as ascorbic acid or ascorbate- is an important dietary antioxidant and it significantly decreases the adverse effect of reactive species such as reactive oxygen species (ROS) that can cause oxidative damage to macromolecules such as lipids, DNA and proteins [15, 17]. Ascorbate is the only antioxidant that completely protects endogenous lipids from detectable oxidative damage induced by ROS [25]. Ascorbate is able to intercept ROS before they can react with and oxidize lipoprotein lipids. Once ascorbate has been depleted, the remaining antioxidants provide only partial protection from ROS, which may interact with lipoproteins and initiate lipid peroxidation. GSH is the most abundant non-protein thiol present in mammalian cells and serves many important physiological roles. This non-protein thiol plays a pivotal role in the protection against ROS in peripheral tissue. Its best known function as an antioxidant is its role as an electron donor in the glutathione peroxidase-catalysed reduction of organic and hydrogen peroxides. Consequently, the oxidized glutathione formed from this reaction removed from the cell via NADPH-dependent reduction by glutathione reductase [8, 19]. In veterinary and human medicine, non-steroidal...
anti inflammatory drugs (NSAIDs) generally are used for the treatment of musculoskeletal and joint diseases and the management of postoperative pain as analgesics. But NSAID therapy, including aspirin, frequently causes gastrointestinal bleeding, erosions and ulceration. Most of the actions may be accounted for by a decrease of endogenous prostaglandins due to inhibition of cyclooxygenase activity [26, 32]. Recently, several reports have suggested that gastrointestinal damage may be initiated by action of NSAIDs on mitochondria [6, 30]. NSAIDs have been reported to uncouple oxidative phosphorylation to dissipate the mitochondrial transmembrane potential [18, 21]. Moreover, the uncoupling effect of NSAIDs was found to induce mitochondrial permeability transition pore [34, 38] leading to the liberation of cytochrome c from mitochondrial intermembranous space into cytosol. It has been reported that liberated cytochrome c generates reactive oxygen species (ROS) such as hydrogen peroxide, thereby causing caspase 9 and caspase 3 activation and cellular lipid peroxidation, all resulting in cellular apoptosis [22].

In this study, we used a canine model of acute injury of the gastric mucosa by using aspirin given orally at a single high dose of 200mg/kg bodyweight and we monitored endoscopic examination, plasma concentrations of MDA, GSH and vitamin C for their relevance of lipid peroxidation in dogs with aspirin-induced mucosal injury.

**Material and Method**

Eight (4 females and 4 males) cross-bred, aging between 8 to 16 months, weighing between 9.2 to 15.3 kg, clinically healthy dogs were used. No infectious or inflammatory processes were previously recorded in those animals. Particularly, dogs were all negative for ehrlichiosis, leishmaniosis, and babesiosis and heartworm disease. Dogs were kept in cooling rooms, in W 1.5m x L 1.0m x H 1.0m cages for the physiological adaptation, fed with commercial dog food and water ad libitum. Dogs were bewareed from any of stress condition such as rapid heat change, noise, high-low moisture and starvation.

At the beginning of the study, clinical examinations and endoscopy were performed just after fasting for 12 hours. Upper gastrointestinal tract was examined using an endoscope (VetVu VFS-2B, Sweden) under 2 mg/kg xylazine HCl (Alfazine, Egevet) and 10 mg/kg ketamine HCl (Alfamine, Egevet) anaesthesia. Following this first examination, aspirin (200 mg/kg bodyweight) was administered orally for once to all dogs [24]. Clinical examinations and endoscopy were repeated and observations were recorded 1, 4 and 7 days after aspirin administration. The presence and localisations of the lesions were recorded using a scale: 0 = no lesion; 1 = non-hemorrhagic erosion in one site; 2 = Non-hemorrhagic erosions more than one site; 3 = Hemorrhagic erosion in one site; 4 = Hemorrhagic erosions more than one site. Experiments were approved by the Animal Ethics Committee of University of Adnan Menderes.

Blood samples were collected from each dog at cephalic vein into tubes containing heparin, before aspirin administration and 1, 4 and 7 days after. Plasma was separated by centrifugation at 1700g for 10 min. All parameters were analysed immediately.

The lipid peroxidation of plasma was measured by the TBA method as described by YOSHIOKA et al [36]. The absorbance of the reaction product of MDA with TBA was measured at 532 nm. Quantification was based upon a molar extinction coefficient of 1.56x10^5M^-1. The plasma GSH concentrations were measured with 0,1mM 5,5’-dithiobis-2nitrobenzoic acid following the method of BEUTLER et al [1]. The amount of reduced product, thionitrobenzene, was measured spectrophotometrically at 412 nm. The plasma ascorbate was measured by the phosphotungtic acid method of KWAY [16].

Results

On the 1st day after aspirin administration, hemorrhagic, linear erosive lesions of the gastric mucosa located always in pylorus and antrum and less frequently in fundus (in 50% of dogs) could be evidenced by endoscopic examination in all dogs (data not shown). At this time, hemorrhagic lesions were relatively widespread throughout gastric mucosa in 63% of dogs. On the 4th day, these lesions seemed to decrease in diameter, were no more hemorrhagic and became focal. Small lesions could not be noticed anymore. On the 7th day, lesions were completely cured in 6 of the 8 dogs used and non-hemorrhagic small and focal lesions were determined in other 2 dogs (Table I). At the 4th and 7th days, the present lesions were mainly localised into the pylorus and the antrum.

<table>
<thead>
<tr>
<th>Dog No</th>
<th>Gastric mucosa lesions (score and localization)</th>
<th>Days post aspirin treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 pa 3pa</td>
<td>1pa 0</td>
</tr>
<tr>
<td>2</td>
<td>0 4pa</td>
<td>2pa 1pa 0</td>
</tr>
<tr>
<td>3</td>
<td>0 3pa f</td>
<td>1pa f 0</td>
</tr>
<tr>
<td>4</td>
<td>0 3pa</td>
<td>1pa 0</td>
</tr>
<tr>
<td>5</td>
<td>0 4pa f</td>
<td>1pa f 0</td>
</tr>
<tr>
<td>6</td>
<td>0 4pa</td>
<td>1pa 0</td>
</tr>
<tr>
<td>7</td>
<td>0 4pa f</td>
<td>1pa 0</td>
</tr>
<tr>
<td>8</td>
<td>0 4pa f</td>
<td>1pa 0</td>
</tr>
</tbody>
</table>

pa = pylorus and antrum; f = fundus
0 = no lesion; 1 = non-hemorrhagic erosion in one site; 2 = non-hemorrhagic erosions more than one site; 3 = hemorrhagic erosion in one site; 4 = hemorrhagic erosions more than one site.

**Table I.** — Characteristics of gastric mucosa lesions observed by endoscopic examination in dogs treated with aspirin (200mg/kg) (n=8).

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On the first day after aspirin treatment, plasma MDA concentrations significantly enhanced (p < 0.01), whereas plasma GSH and vitamin C concentrations were markedly lowered (p < 0.01). A negative correlation between vitamin C and MDA concentrations was obtained at this time (r = -0.39; p < 0.05). Thereafter, on the 4th day, the blood concentrations and MDA concentrations was obtained at this time (r = -0.39; p < 0.01). Thereafter, on the 4th day, the blood concentrations and MDA concentrations was observed on the 1st day.

Discussion

Aspirin at a dose of 200 mg/kg have been found suitable to induce gastric injury in rats by DEBASIS BAGCHI et al. [7]. The researchers have examined the effects of aspirin in rats on lipid peroxidation, membrane microviscosity and DNA fragmentation in gastric and intestinal mucosa and have reported that aspirin produced a 2.0 to 3.7 fold increased lipid peroxidation. The present study indicated that high dosage of aspirin induced gastric injury and caused accelerated lipid peroxidation in blood of dogs. It is generally accepted that NSAIDs exert pro-ulcerogenic activity related to their ability to inhibit endogenous prostaglandin synthesis. Consequently, the gastric acid secretion increases, whereas non-parietal components of gastric secretion such as sodium bicarbonate and mucus decrease and the mucosal blood flow is reduced [2, 31, 32]. The focal ischemic areas, which subsequently develop into erosions and inadequate perfusion, may disturb cell metabolism with local release of tissue-damaging mediators such as oxygen-derived free radicals. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of the injury of various tissues, including the digestive system [4, 26]. Lipid peroxidation by oxygen radicals is believed to be an important cause of cell membrane damage, because a single initiating event in which a hydroxyl radical or metal ion-free radical complex removes methylene hydrogen atoms from polyunsaturated fatty acids can result in the conversion of hundreds of fatty acid side chains into lipid peroxides, altering the structural integrity and biochemical function of membranes [11, 23]. In the present study, the increases of plasma MDA concentrations and the decreases of plasma vitamin C and GSH concentrations observed on the 1st day evidenced the induction of an oxidative stress by aspirin administration, and the marked consumption of the antioxidant compounds, due to their direct roles for neutralizing ROS (Vitamin C and GSH) or for recycling the antioxidant capacities (GSH). Nevertheless, as plasma MDA, vitamin C and GSH concentrations have significantly changed and gastric mucosal damage have markedly reduced on the 4th day, the oxidant effects of aspirin were transient, probably because of the short half-life of this drug. The relationship between NSAID-induced gastric mucosal lesions and lipid peroxidation in gastric tissue evidenced by the MDA production and the depression of polyunsaturated fatty acids which serve as substrates for radical attack [3, 20] as well as reduced activities of glutathione peroxidase [35], superoxide dismutase [37] and catalase, and the decreases of glutathione concentrations [13] has already been established. Altered glutathione homeostasis and enzymatic antioxidant defence, together with decrease of PGE2 production, were also observed after meloxicam treatment [33].

In healthy animals, a large part of cellular metabolic activity is normally devoted to reduction processes that combat the threat of oxidation. Glutathione is involved in defence processes against oxidative damage [23, 29]. In view of importance of glutathione redox cycle defence system against peroxides, it is not surprising that any deficiency of these antioxidants is likely to impair the protective system increasing the cellular susceptibility to oxidative damage [29].

Vitamin C present in aqueous environment has multiple antioxidant properties including the ability to regenerate α-tocopherol by reducing α-tocopherol radicals present on the surface of membranes [10]. We have observed a decrease of vitamin C concentrations in aspirin induced gastric injury. This decrease could result from an increased utilization of vitamin C as direct antioxidant against increased ROS, or from an insufficient vitamin C recycling by GSH due to decreased GSH concentrations.

Despite of the small number of animals used, this study remains to be exclusive in dogs where the MDA and vitamin C/GSH concentrations were simultaneously measured. Moreover, as the aspirin-induced gastric injury develops, MDA versus antioxidant (Vitamin C and GSH) concentrations shifts in the opposite direction and this fact suggests that aspirin has induced lipid peroxidation which in turn has lead to consumption of antioxidant systems. It would be useful to consider anti-oxidant drugs in the treatment of acute

Table II. Plasma MDA, vitamin C and blood GSH concentrations in dogs treated with aspirin (200mg/kg) (n=8) before (day 0) and 1, 4 and 7 days after drug administration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>1</th>
<th>4</th>
<th>7</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/L)</td>
<td>12.40 ± 2.4</td>
<td>21.00 ± 3.2</td>
<td>12.34 ± 1.02</td>
<td>11.01 ± 3.40</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GSH (g/L)</td>
<td>2.73 ± 0.25</td>
<td>17.19 ± 0.24</td>
<td>2.00 ± 0.34</td>
<td>2.47 ± 0.32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vitamin C (mg/L)</td>
<td>206 ± 20</td>
<td>72 ± 9</td>
<td>106 ± 7</td>
<td>175 ± 18</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

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7. DEBASHIS BAGCHI, PhD, OWEN R. CARRYL, PhD, MINH X. TRAN, MANASHI BAGCHI, PhD, PHILLIP J. VUCHEITCH, ROGER L. KOV, SIDDHARTH A. D. RAY, PhD, SEKHAR MITRA, PhD, and SIDNEY J. STOHS, PhD : Protection against chemically-induced oxidative gastrointestinal tissue injury in rats by bis-naphthol salts. *Dis. Dig. Sci.*, 1997; 42 (9) : 1890-1900.


Gastritis in order to decrease the oxidative stress. The dose / effect relationship between aspirin treatment, oxidative stress and antioxidant status and upper gastrointestinal tract injury may be a further point of investigation.