Disturbance of oxidant/antioxidant balance, acute phase response and high mobility group box–1 protein in acute undifferentiated diarrhea in crossbred piglets

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Piglet diarrhoea is a major cause of high morbidity and mortality in the swine production farm, resulting in significant magnitude of economic losses. The common enteric pathogens responsible for piglet diarrhoea are virus (transmissible gastroenteritis, porcine epidemic diarrhoea virus, porcine circovirus-2 and rotavirus), bacteria (enterotoxigenic Escherichia coli, Salmonella sp., Clostridium perfringens), and parasites (Isospora suis and Cryptosporidium parvum) apart from many other pathogens (Katsuda et al. 2006). Release of some molecules following disruption of intestinal mucosa aggravates the pathogenesis of enteritis. The role of high mobility group box 1 (HMGB1) protein, which is released in response to activated signals by pathogen derived toxins and virus stimuli (Wang et al. 2006), in the pathogenesis of gastrointestinal disorders was investigated (Dai et al. 2010, Luan et al. 2010). The acute phase proteins are considered to be involved in the restoration of homeostasis and restraint of microbial growth before animals develop acquired immunity to infection (Petersen et al. 2004). Haptoglobin (Hp) and ceruloplasmin (Cp), the porcine acute phase proteins, are well studied in piglet (Zyczko and Zyczko 2005, Grau-Roma et al. 2009). Kim et al. (2012) suggested that tissue damage attributed by oxidative stress and reactive oxygen species (ROS) plays a key role in pathogenesis in enteric diseases of farm animals. The nitric oxide (NO) contributes to oxidative stress in conjunction with other ROS in the states of inflammation (Lubos et al. 2008). To the best of our knowledge, there are no such reports and detail studies describing the role of HMGB1, acute phase proteins and oxidative stress in acute undifferentiated piglet diarrhea. Therefore, the present study was undertaken to examine whether acute undifferentiated diarrhea influences these levels in crossbred suckling piglets and that these may be targets for supportive therapy.

Animals: Crossbred piglets (Landrace × indigenous) from the institute’s experimental swine production farm were included for the present study. The piglets and dam were kept in same farrowing pens with concrete floor and piglets were allowed to suck ad lib dam’s milk to the age of 7 weeks. The overall management practices were identical for all piglets. The pregnant dams were not immunized with E. coli K99+ vaccine and any viral vaccine, nor were additional supplementation of antioxidant practiced in the farm. Fifteen piglets between 0–15 days old of age, irrespective of sex and body weight, that were suffering from acute diarrhoea were randomly used for sampling. The diarrhoea was diagnosed on the basis of clinical symptoms such as frequency of defecation (>5 times in a day), consistency (profuse, watery, with or without mucus), dehydration score (prominent protruding vertebrae and pelvic bones and retracted eyes), dullness and weakness. Another 15 healthy piglets of either sex of same age group were also randomly selected to serve as controls.

Collection and processing of samples: Blood samples (5 ml) from both groups of piglets were collected from anterior vena cava in sterile vials containing disodium salt of ethylene diamine tetraacetic acid (Na2-EDTA) as anticoagulant. Samples were immediately centrifuged at 200g for 10 min to separate plasma and stored at -20°C till analysis.

Oxidative stress markers

Assay for malondialdehyde (MDA) and nitric oxide (NOx): The MDA levels, an index of lipid peroxidation were measured by double heating method of Draper and Hadley (1990). The assay is based on spectrophotometric measurement of the purple colour generated by the reaction of thiobarbituric acid (TBA) with MDA. The concentration of MDA was calculated on the basis of absorbance coefficient (1.56 × 105 cmM–1) of the TBA-MDA complex. The nitrite and nitrate (NOx) in plasma was measured by nitrate reduction on vanadium chloride followed by colour
development with Griess reagent as per the method described earlier (Yucel et al. 2012).

Assay for total antioxidant activity, superoxide dismutase (SOD) and catalase: Total antioxidant, superoxide dismutase (SOD) and catalase activity in blood serum were estimated by antioxidant assay kit, SOD assay kit and catalase assay kit respectively as per manufacturer’s instruction.

Assay for high mobility group box–1 (HMGB1) protein, haptoglobin (Hp) and ceruloplasmin (Cp): Before analysis, all the plasma samples were diluted into 1:10 and HMGB1 protein in diluted serum was estimated using HMGB1 ELISA kit according to the manufacturers’ instructions. Similarly, Hp was measured in diluted plasma samples (1:40,000) using Assay swine haptoglobin ELISA kit as per the manufacturers’ instructions. All ELISA measurements were performed in duplicates, and measured at 450 nm with the Multiskan RS Microplate Reader. The Cp level in serum was determined by measuring paraphenylene diamine (PPD) oxidase activity as per Sunderman and Nomoto (1970).

Statistical analysis: The data were analyzed statistically using Student’s t-test (P<0.05) to determine the significance between the mean values of the two groups (Snedecor and Cochran 1994).

Oxidative stress is manifested either due to inadequate availability of antioxidant enzymes or uncontrolled production of free radicals in the body. Free radicals and lipid peroxidation exert harmful effect on tissue due to alternations in the integrity of cell membrane (Lobo et al. 2010). MDA, derived from lipid peroxidation is an important indicator to decide the degree of oxidative damage of cell membrane. It reacts with cellular membrane elements and results in increase cellular permeability and enzyme activities. NO is considered as an important component of the host defense against invading pathogens. This molecule, a principal endothelial-derived relaxing factor, reacts with superoxide anion (O2-) to yield peroxynitrite (ONOO-), which is a powerful oxidant and nitrosating agent. It triggers a cascade of events leading to the generation of highly reactive and damaging radicals and oxidative species (Soneja et al. 2005). Body counteracts the ill effects of oxidative damage by neutralizing the free radicals via antioxidant defense system that comprises antioxidant enzymes, like superoxide dismutase (SOD) and catalase (CAT). In the present study, serum MDA (7.523±0.451 nmol/ml vs 2.455±0.182 nmol/ml) and NOx (4.004±0.224 μmol/min/ml vs 2.104±0.173 μmol/min/ml) were significantly (p<0.05) increased and total antioxidant activity, (1.766±0.160 Trolox mEq/ml vs 3.856±0.112 Trolox mEq/ml), SOD (143.251±19.498 U/ml vs 336.547±9.462 U/ml) and CAT (6.195±0.532 formaldehyde nmol/min/ml vs 16.430±2.155 formaldehyde nmol/min/ml) level were decreased (p<0.05) in diarrheic piglets as compared to control animals (Table I). It might be due to excess production of free radicals and poor antioxidant reserve in piglets suffering from acute diarrhoea. Authors reported that enhanced activity of oxidative stress markers in pigs experimentally infected with ETEC E. coli, Cryptosporidium parvum and other enteric pathogens (Argenzio and Rhoads 1997, Keel and Songer 2006, Zadrozny et al. 2006, Daudelin et al. 2011). The present finding warrants the need of antioxidant supplementation with standard treatment for better therapeutic response in acute undifferentiated piglet diarrhea.

The HMGB1 is released from necrotic cells during tissue damage as a consequence of infection and leads to leakage of epithelial-cell barriers because of down regulation of expression of some cell-surface proteins responsible for the tight adhesion between adjacent epithelial cells (Kono and Rock 2008). This cellular mechanism is also responsible for the systemic and organ-specific toxicity of HMGB. In the present study, significantly (p<0.05) higher levels of serum HMGB1 (6.629±0.367 ng/ml vs 2.350±0.065 ng/ml) in diarrheic piglets as compared to control animals (Table I) might be due to excessive damage of intestinal epithelial cells in piglets suffering from acute enteritis. Increased HMGB1 activity has been reported in acute gastroenteritis induced by E. coli, Shigella dysenteriae in piglet model and in children with inflammatory bowel disease (Jeong et al. 2010, Splichalova et al. 2011, Vitali et al. 2011). However, it has been reported that the lethal effect caused by HMGB1 protein in gut could be neutralized using HMGB1-specific antibodies (Yang et al. 2004). There is no published report on HMGB1 protein in natural cases of piglet diarrhea. From the present study, we hypothesize that serum HMGB1 could be used as a reliable clinical evaluation tool of the severity of enteric infections in piglets.

The acute phase response is a nonspecific reaction to tissue damage by infection, inflammation, neoplasia and immunological disease (Chen et al. 2003, Petersen et al. 2004). It triggers the production of acute-phase proteins (APPs) (such as α-1 acidglycoprotein, haptoglobin and ceruloplasmin) and involves local and systemic effects (Green and Adams 1992). Moreover, the APPs are also the

Table 1. Comparison of parameters of oxidative stress markers, high mobility group box–1 protein and acute phase response in crossbred piglets affected with acute undifferentiated diarrhoea and healthy piglets

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy piglets</th>
<th>Diarrheic piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant activity (trolox mM/ml)</td>
<td>3.856±0.112</td>
<td>1.766±0.160*</td>
</tr>
<tr>
<td>Catalase (Formaldehyde nmol/min/ml)</td>
<td>16.430±2.155</td>
<td>6.195±0.532*</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>336.547±9.462</td>
<td>143.251±19.498*</td>
</tr>
<tr>
<td>MDA (nmolml–1)</td>
<td>2.455±0.182</td>
<td>7.523±0.451*</td>
</tr>
<tr>
<td>NOx (μmol/ml)</td>
<td>2.104±0.173</td>
<td>4.004±0.224*</td>
</tr>
<tr>
<td>HMGB1 (ng/ml)</td>
<td>2.350±0.065</td>
<td>6.629±0.367*</td>
</tr>
<tr>
<td>Hp (ng/ml)</td>
<td>0.455±0.064</td>
<td>2.415±0.103*</td>
</tr>
<tr>
<td>Ceruloplasmin (g/litre)</td>
<td>0.213±0.019</td>
<td>0.605±0.039*</td>
</tr>
</tbody>
</table>

*Statistically significant at P < 0.05.
components of innate immunity mediated by cytokines (Suffredini et al. 1999). In the present study, serum Hp (2.415±0.103 ng/ml vs 0.455±0.064 ng/ml) and ceruloplasmin (0.605±0.039 g/litre vs 0.213±0.019 g/litre) level were significantly (P<0.05) higher in piglets suffering from acute diarrhea as compared to healthy piglets. Over the past few years, intestinal epithelial cells have been shown to play a central role in the inflammatory response of the intestine in response to enteric pathogen (Stadnyk and Waterhouse 1997). Researchers observed that genes of acute phase proteins are expressed and regulated by multiple cytokines and cAMP in intestinal epithelial cells (Pelletier et al. 1998). The enteric pathogens stimulate the synthesis of pro and anti-inflammatory cytokines and activity of cAMP in intestinal epithelial cells during infection (Moen et al. 2010, Shea-Donohue et al. 2010). Significant elevation of Hp and ceruloplasmin in diarrheic piglets as compared to healthy animals in the present study could be due to inflammation and intestinal tissue injury caused by enteric pathogens.

From the present findings, it can be concluded that oxidative stress, HMGB1 and acute phase response play a definite role in the pathogenesis of piglet diarrhea, suggesting a possible beneficial role for HMGB1 specific antibody and supplementation of antioxidants along with standard treatment in piglet diarrhea.

SUMMARY

The objective of the present study was to investigate the status of high mobility group box-1 (HMGB1) protein, oxidative stress and acute phase proteins in natural cases of acute undifferentiated diarrhoea in piglets aged 1–15 days old. The study was conducted on 30 crossbred (Landrace × indigenous) piglets; fifteen suffering from acute enteritis (group 1) and fifteen healthy piglets as control (group 2). The diarrhoea was diagnosed on the basis of clinical symptoms. From the results of the study, it is concluded that HMGB1 protein, markers of oxidative stress and acute phase proteins might play important roles in the pathophysiology of piglet diarrhoea and that these may be targets for supportive therapy.

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REFERENCES


