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Research paper

Immunomodulatory potential of β-glucan as supportive treatment in porcine rotavirus enteritis

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ABSTRACT

A non-blinded randomized clinical trial was conducted to assess the immunomodulatory potential of β-glucan (BG) in piglet diarrhoea associated with type A rotavirus infection. A total of 12 rotavirus-infected diarrheic piglets were randomly divided into two groups: wherein six rotavirus-infected piglets were treated with supportive treatment (ST) and other six rotavirus-infected piglets were treated with BG along with ST (ST-BG). Simultaneously, six healthy piglets were also included in the study which served as control. In rotavirus-infected piglets, marked increase of Intestinal Fatty Acid Binding Protein-2 (I-FABP2), nitric oxide (NOx), Interferon-γ (IFN-γ) concentrations and decrease of immunoglobulin G (IgG) were noticed compared to healthy piglets. The faecal consistency and dehydration scores were significantly higher in rotavirus-infected piglets than healthy piglets. The ST-BG treatment progressively reduced the I-FABP2 and increased the IgG concentrations over the time in rotavirus-infected piglets compared to piglets received only ST. A pronounced enhancement of NOx and IFN-γ concentrations was observed initially on day 3 and thereafter the values reduced on day 5 in ST-BG treated piglets in comparison to piglets which received only ST. Additionally, ST-BG treatment significantly reduced faecal consistency and dehydration scores on day 3 compared to ST in rotavirus-infected piglets. These findings point that BG represents a potential additional therapeutic option to improve the health condition and reduce the piglet mortality from rotavirus associated diarrhoea where porcine rotavirus vaccine is not available.

1. Introduction

Rotavirus is the foremost cause of gastrointestinal infections in mammals all over the world (Malik et al., 2014). Rotaviral enteritis causes significant magnitude of economic loss in the swine industry worldwide by increasing cost of treatment, prevention and potentiating loss of stock quality through disease and/or death (Gachanja et al., 2015). Although all age groups of pig are susceptible, rotavirus infection is most common in young suckling piglets (Dewey et al., 2003). Differentiated intestinal epithelial cells, primarily the epithelial cells of the ileum and jejunum, are the main targets of rotavirus (Ward et al., 1996). The virus damages the villi of the small intestine through detachment of enterocytes from the lamina propria, leaving denuded areas and marked villous atrophy (Torres-Medina and Underdahl, 1980; Zijlstra et al., 1997; Ciarlet and Estes, 2001). Fatty acid binding protein (FABP), an intracellular protein with a low molecular weight of approximately 15 kDa, has major role in the transportation and metabolism of long-chain fatty acids (Funaoka et al., 2010). Among the FABP family proteins, intestinal fatty acid-binding protein-2 (I-FABP2) is specifically and abundantly present in epithelial cells of the small intestinal tissue and a certain amount is found in epithelial cells of stomach (Sacchettini et al., 1987). The concentration of I-FABP2 is very negligible in the serum, but it rapidly releases into the circulation following small intestinal tissue injury or intestinal ischemic injury (Windsant et al., 2012; Uzun et al., 2014).

In any viral infection, the stimulation of innate immune system of host is very crucial to prevent viral invasion or replication and activation of adaptive immunity (Takeuchi and Akira, 2009). Like other viruses, rotavirus elicits innate and acquired virus-specific humoral and cellular immune responses (Holloway and Coulson, 2013). In the innate immune response, pattern recognition receptors are engaged to detect specific viral components or its intermediate products and induce interferons and other pro-inflammatory cytokines in the infected cells and immune cells (Koyama et al., 2008). Although both IFN-α/β and IFN-λ

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are likely to play important roles in response to rotavirus infection, their relative contributions depend on the synergistic effects of IFN-γ (Arnold et al., 2013). It has been reported that the rotavirus-encoded non-structural protein 1 blocks and down regulates interferon production by various pathways (Barro and Patton, 2005, 2007; Aich et al., 2007). NOx, a messenger molecule, has a complex role in immunological host responses against viruses. The antiviral effects of NOx contribute to innate immunity of host against viruses and are mediated by polarized Th1 ± Th2 immunological reactions. NOx impairs antiviral response by suppressing Th1 (particularly IFN- γ production) functions (Akaike and Maeda, 2000). Previous workers observed that non-structural protein 4 of rotavirus triggers the production of NOx by macrophages through Toll-like receptor 2 and possibly is augmented by secreted IFN-γ (Borghan et al., 2007). Ge et al. (2013) reported that induction of NOx is a mechanistic link to the induction of diarrhoea.

In absence of any suitable antiviral agents to protect the piglets against this disease, symptomatic treatment is the only choice that is available to reduce the morbidity and mortality due to rotaviral piglet diarrhoea. Several studies have explored the use of immunomodulators as therapeutics to modify the immune response to virus infection, thereby preventing or decreasing viral burden, disease symptoms, and mortality (Norton et al., 2010). Recently, certain *Lactobacilli* and *Bifidobacterium* strains exhibited anti-rotaviral effects due to their modulation of the immune response in mouse and gnotobiotic porcine models (Vlasova et al., 2013; Liu et al., 2014; Kang et al., 2015). Beta-glucan, a diverse class of glucose polymers, has received attention owing to its effective immunomodulatory effects (Zeković et al., 2005; Baert et al., 2015). Beta-glucan, a carbohydrate, is consisted of linked glucose molecules and the major structural component of the cell walls of yeast, fungi and some bacteria (Julia et al., 2008; Du et al., 2013). The biological activity of β-glucan is greatly influenced by source, degree of branching, size and molecular structure (Zhang et al., 2005; Leung et al., 2006; Volman et al., 2008). The biologically most active form of β-glucan possesses a common structure consisting of a main chain of (1–3)-linked β-D-glucopyranosyl units along which are randomly dispersed β-D-glucopyranosyl units attached by 1–6 or 1–4 linkages (Bohn and BeMiller, 1995; Zeković et al., 2005). Besides the source and length, the frequency of the branches varies depending on the source of β-glucan (Yoshitomi et al., 2005; Leung et al., 2006). Previous researchers reported that the molecules of β-glucan having higher ordered structures of triple helical are responsible for the immunomodulatory activity (Zhang et al., 2005; Batbayar et al., 2012). Earlier studies on relationship between molecular structure and bioactivities of glucans indicate that water solubility, rigidity of chain and helical chain conformation are beneficial for antivirus activities of polysaccharides (Rouhier et al., 1995; Wang et al., 2017). The recognition of β-glucan by immune cells via several pattern recognition receptors, including complement receptor 3 (CR3), dectin-1, lactosylceramide and scavenger receptor is very crucial for its activity (Janeway, 1992; Soltanian et al., 2009). The β-glucan can stimulate the host immune system through modulation of humoral and cellular immunity and shows beneficial effect against infectious agents such as bacteria, virus, fungi and parasites (Mantovani et al., 2008). In porcine, β-glucan binds with CR3 and dectin-1 receptors of innate immune cells and results to an enhancement of pro-inflammatory cytokine production, phagocytosis, chemokines and oxidative burst activity (Baert et al., 2015). It is extensively used in human medicine because of its immunomodulatory, anti-cytotoxic, anti-mutagenic, hypoglycaemic, anti-microbial and anti-tumorogenic activities (Huff et al., 2006; Mantovani et al., 2008). The anti-viral effect of *Saccharomyces cerevisiae* derived β-glucans to swine influenza virus has been reported (Jung et al., 2004; Wang et al., 2008). However, its immunomodulatory effect has not been explored in rotavirus associated diarrhoea of piglets. Therefore, in this study we examined whether β-glucan can stimulate certain innate immune indicators and reduce intestinal injury markers using porcine rotavirus enteritis model.

2. Materials and methods

2.1. Care and use of animals and experimental design

The care of selected animals was undertaken as per the guidelines of the Institutional Animal Ethics Committee and the protocol of the experiment (No. F.25/08/2016-CPCSEA) was approved by Committee for the Purpose of Control and Supervision of Experiments on Animals, India. The study was conducted at Swine Production Farm, Livestock Production Management Section, Indian Veterinary Research Institute, Izatnagar (India) during November 2015–May 2016. A total 47 piglets (age group: 0–2 weeks) irrespective of sex and body weight, suffering from diarrhoea, were randomly used for sampling and screening for rotavirus infection. The diarrhoea was diagnosed on the basis of clinical symptoms such as frequency of defecation (> 5 times in a day), consistency of stool (profuse, watery, with or without mucus), status of dehydration (severe, prominent protruding vertebral and pelvic bones and eyes retracted in orbit > 1 mm), dullness and weakness. The status of dehydration of piglets showing diarrhoea was assessed as per the method described by Pereira et al. (2002). Of these 47 faecal samples, 21 were found positive for rotavirus infection by VP6 gene based reverse transcription-polymerase chain reaction (RT-PCR). Out of 21 rotavirus-positive diarrheic piglets, 12 rotavirus-positive piglets were randomly picked up and divided equally into two treatment groups, each group consisting of 6 rotavirus-positive diarrheic piglets. Six rotavirus-positive piglets received supportive treatment (ST) consisting of antibiotic (Ceftriaxone @ 10 mg/kg body weight, intramuscular route, twice a day), fluid (25.0 mL Ringer's lactate in 3 divided doses orally per day) as well as anti-inflammatory drug (Meloxicam @ 0.4 mg/kg body weight, intramuscular route, once a day) when indicated, whereas, another six rotavirus-positive piglets received ST plus β-glucan (ST-BG) (Beta Glucan, β1,3/β1,6 branched glucan extract (purity > 80%) of the yeast (*Saccharomyces cerevisiae*) cell walls, Jarrow Formulas, Los Angeles, CA) @ 50 mg/Kg body weight once daily orally for 5 consecutive days. One more group consisting of six healthy piglets was kept for the study, which served as control. All the piglets were treated after examination by expert clinician of the institute.

2.2. Diagnosis of porcine type A rotavirus

The faecal samples from diarrheic piglets were collected in a sterile container (Sterile clinicol TM, Himedia laboratories Pvt. Ltd. Mumbai, India) and transported on ice to laboratory for diagnosis of rotavirus infection. Screening of rotavirus in faecal samples was carried out by VP6 gene based RT- PCR.

A 10% (w/v) faecal suspension of the faecal material was prepared with sterile phosphate buffered saline (PBS; pH 7.2) by dissolving 0.1 g of faeces in 1.0 mL PBS. After thorough vortexing, centrifugation (Eppendorf, Centrifuge 5804R, Eppendorf AG, Hamburg, Germany) was carried out at 2655*g* for 5 min to remove the coarse debris. The RNA was extracted from supernatant using QIAzol Lysis Reagent (Cat. No. 79306, QIAGEN, Hilden, Germany). For reverse transcription, 1.0 μL (0.4 μg/mL) of random hexamers, 0.70 μL of DMSO (dimethyl sulfoxide), 15.0 μL (57 ng/μL) of extracted RNA and 1.0 μL of Nuclease Free Water (NFW) were mixed. The reaction mixtures was denatured at 95 °C for 5 min in dry bath and immediately snap chilled on ice. The remaining reagents, which consisted of 5 μL of 5× RT buffer (50 mMTris-HCl, 75 mM KCl, 3 mMMgCl2), 1.0 μL of dNTP 10 mM (deoxynucleotide triphosphate), 0.30 μL of ribolock and 1.0 μL (50 U/μL) M-MLV RT enzyme, were added to the tube to make it into the total volume of 25.0 μL. The mixture was incubated at 37 °C for 1 h, and the reaction was stopped by heating to 80 °C for 5 min to inactivate residual MMLVRT. The complimentary DNA (cDNA) was either stored at −20 °C or amplified immediately. Diagnostic PCR was initiated by using internal primers, which were directed towards the group specific protein gene VP6 to identify the type A