Journal of Equine Veterinary Science 34 (2014) 1156–1163

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Orally Administered *Pediococcus acidilactici* and *Saccharomyces boulardii*–Based Probiotics Alter Select Equine Immune Function Parameters

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ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 14 June 2014
Accepted 24 June 2014
Available online 28 June 2014

Keywords:
Equine
Probiotics
Saccharomyces boulardii
Pediococcus acidilactici
Immune response

ABSTRACT

To investigate the effects of a combination of *Pediococcus acidilactici* and *Saccharomyces boulardii*, the following experiments were performed. Initially, an in vitro experiment was performed in which the culture supernatant of *S. boulardii* and *P. acidilactici* was added to the culture media of isolated peripheral blood mononuclear cells (PBMCs), and the proliferative response to cellular stimulants was assayed. After this initial experiment, an in vivo experiment was performed in which 12 horses were used and assigned to one of two groups of six horses each: placebo controls or principle-treated horses. After a period of treatment, various end points were determined to test the effects of test article on (1) proliferative responses of cultured PBMCs; (2) serum immunoglobulin (Ig) concentrations; (3) lymphocyte phenotype subsets; (4) white blood cell count; and (5) response to vaccination. Results of the in vitro testing demonstrated a substantial reduction (23%) in proliferation of stimulated PBMCs. Results of in vivo testing demonstrated enhanced proliferation on day 72 in cells stimulated with phorbol ($P = .04$). On study day 37, the segmented neutrophil number was reduced and IgG concentration increased (mean, 329.0 vs. 185.9 ng/mL; $P = .029$). Results demonstrate that the test article did have some effects on systemic immunity, specifically proliferative responses, immunoglobulin G concentrations, and neutrophil numbers. Based on the findings of this study, further evaluation of these probiotics for equine wellness or disease modulation is warranted.

1. Introduction

Immunomodulators are defined as substances that produce non–antigen-specific enhancement of the bodies' native defense mechanisms; they have also been termed "biological response modifiers" [1]. Immunomodulators can be used either to enhance normal immunity (immunostimulants) and suppress overexuberant immune response (immunosuppressants) or to restore deficient immunity.

A particular subtype of immunomodulators are the "probiotics." According to the World Health Organization, probiotics are defined as "live (nonpathogenic) microorganisms that confer a health benefit to the host when administered in adequate amounts" [2]. Among these effects, the control or prevention of serious gastrointestinal illnesses are considered to be particularly important and amenable to modulation by orally administered probiotic compounds [2]. A number of yeasts and lactic acid bacterial products have been described and used as probiotics in various species [3–12].

Two organisms that have been investigated in recent years, and which show promise as probiotics, include *Saccharomyces boulardii* and *Pediococcus acidilactici*. *Saccharomyces boulardii*, a subtype of the nonpathogenic

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http://dx.doi.org/10.1016/j.jevs.2014.06.020

yeast *Saccharomyces cerevisiae*, is nonpathogenic and resistant to antibiotics, is acid tolerant, and grows at the relatively high temperature of 37°C [13]. *Saccharomyces boulardii* has been used for the treatment of diarrhea in humans for many years, and has been studied in the horse with variable results [4,5,14–17].

Pediococcus products have not been formally tested in the horse. It has been suggested that horses treated with a combination of *P. acidilactici* and *S. boulardii* had enhanced clearance of intestinal parasites and improved fecal consistency [18]. Based on this observation and research in other species, the effect of *P. acidilactici and S. boulardii* (administered together as a capsule) on selected parameters of equine immune function were evaluated. The hypothesis tested was that treatment with the probiotic organisms would have no effect on assayed immune function parameters. The protocol was reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee.

2. Materials and Methods

2.1. In Vitro Study

Initially, the in vitro proliferative response of cultured peripheral blood mononuclear cells (PBMCs) was tested in the presence of either 5% or 20% (vol/vol) *P. acidilactici* and/or *S. boulardii* culture supernatant (pooled 1:1; Imagelin Technologies, Frederick, MD). Blood was collected from six normal healthy horses by jugular venipuncture; horses were considered to be clinically healthy based on physical examination and a normal complete blood cell count (CBC) performed immediately before blood collection. Peripheral blood mononuclear cells were harvested using density gradient centrifugation (Lymphoprep, SG 1.077; Nicomed, Oslo, Norway). Cells were counted, and an aliquot was stained for viability using vital dye exclusion (Trypan blue 0.4% solution, Sigma-Aldrich, St Louis, MO); 2×10^5 viable cells were suspended in 300 mL of complete media (Rosewell Park Memorial Institute with 10% vol/vol fetal calf serum, 2.0 mM L-glutamine, gentamicin 5 μL/mL and 100 U/mL penicillin, and 20 mM HEPES; Sigma-Aldrich) containing either 5% or 20% (vol/vol) of culture supernatant from *P. acidilactici* and/or *S. boulardii* cultures (Imagelin Technologies). Treatments were replicated in triplicate. Cellular proliferation was determined using the alamar blue dye reduction method, as previously reported [19]. Cells were stimulated by the addition of either 5 or 20 μg/mL of concanavalin A (Con A) or phorbol myristate acetate (PMA) (20 μg/mL) and ionomycin (10 pg/mL) (Sigma-Aldrich). Cells were incubated at 37°C in 5% CO_2 (humidified) for 60 hours, then 20 μL of alamar blue (Alamar Blue 10X ready-to-use solution, Invitrogen, Eugene, OR) was applied, and the absorbance at 590 nm was read after an additional 2 hours. Increasing absorbance is directly related to increased cellular proliferation.

2.2. In Vivo Study

Twelve normal healthy adult horses were used in the in vivo part of the study and were randomly assigned to one of two treatment groups: (1) treated with *P. acidilactici* and *S. boulardii* using a commercially available product with one billion live particles per capsule (MitoHorse; Imagelin Technologies) or (2) placebo capsule with sucrose. Horses were determined to be clinically normal based on a routine clinical examination and clinicopathologic examination (CBC and routine serum chemistry analysis). In addition to physical examination, resting serum cortisol concentrations were determined to screen for the presence of posterior pituitary adenoma, a condition which was considered to potentially confound the results if present. Horses were excluded from the study if the resting serum cortisol concentration (from a morning sample) was higher than the accepted normal range from the testing reference laboratory (Cornell University Diagnostic Laboratory; $N = 2–6$ μg/dL). In addition, if horses had clinical signs consistent with pituitary adenoma (hirsutism, abnormal fat distribution, polyuria and/or polydipsia, and so forth) laminitis, abnormal white blood cell count, increased fibrinogen, or any clinical abnormality that might interfere with completion of the study they were excluded. Horses

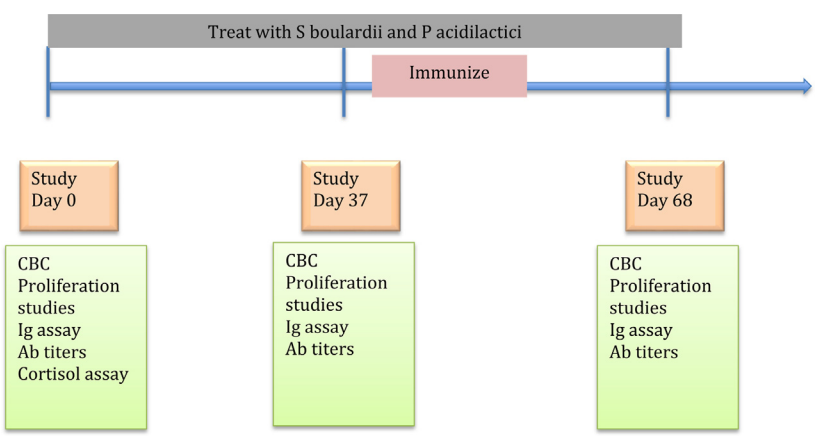

Fig. 1. Timeline for study with critical time points indicated. Ab, antibody; CBC, complete blood cell count; Ig, immunoglobulin; *P. acidilactici*, *Pediococcus acidilactici*; *S. boulardii*, *Saccharomyces boulardii*.