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Full Length Research Paper

The protective effect of beta glucan against *Escherichia coli* infected mice via intraperitonial administration

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Mice infected with *Escherichia coli* were protected against lethal peritonitis by the intra peritoneal administration 0.2 ml of 10 mg of poly-1,3,1,6-glucotriosyl-1,3,1,3-glucopyranose (PGG) glucan per gram body weight of animal 24 h prior to bacterial infection. This procedure employed in this study, is similar to that employ in a previous studies where rat model were protected with β-glucan against intra-abdominal sepsis, where the intramuscular doses of 10 ml to 10 g per animal 24 h and 4 h prior to surgical implantation of the bacterial inoculums reduced the early mortality associated with the peritonitis phase of this experimental disease process. In this study mice were protected with β-glucan against peritonist 24 h prior to intraperitoneal administration of bacteria inoculums. Quantitative cultures of the liver obtained from mice infected with *E.coli* showed significantly the liver of PGG glucan-treated infected animal has a reduced microbial load as compare to those infected and were not treated. The transient increase in survival rate of mice infected with *E.coli* that have been treated with PGG glucan- as compare with that of those not treated shows the significant effect of β glucan on *E.coli* infection.

Key words: Beta-glucan, *Escherichia coli,* poly-131-6-glucotriosyl-j31-3-glucopyranose (PGG) glucan, peritonitis, inoculums, intra-peritoneal, intramuscular

INTRODUCTION

The discovery of zymosan a crude insoluble extract from yeast cell wall, as a stimulant, that has an ability to stimulate the reticuloendothelial system initiate the identification of the immunological properties of yeast, (Riggi and DiLuzio, 1961; Ondoderk et al.,1992). Further study was able to identify the active moiety of the yeast cell wall as beta-glucan (Bacon et al., 1960; Phaff, 1963; Onderdonk et al., 1992).

Beta glucan had been described to be able to stimulate the immune system, owing to its ability to increase the plasma level and splenic level of Interleukin 1 and 2 in rat (Czop and Austen, 1985).

The immune modulating properties of β-glucan had been related to its ability to bind to specific receptor site of the human neutrophils and macrophages (Czop and Austen, 1985).

It has been shown that host defense responses related to the β-glucans include the activation of the alternative complement pathway, the release of lysosomal enzymes by monocytes, and the generation of leukotrienes by monocyte (Czop et al., 1978; Czop and Austen, 1985; Janusz et al., 1987). Immune modulators are known to exhibit their activities in animal by increasing the non specific component of the immune system such as the macrophages, and neutrophil, and can induce innate immune response by stimulating the maturation of the myeloid progenitor cells (Kounnikasis et al., 2003). This ability of immune modulators such as β-glucan to activate macrophages is considered the first line of defense against microorganism. Once the macrophages are activated they have the ability to stimulate the secondary

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line of defense, which is the effective humoral and cellular response (Abass et al., 2000).

The ability of β-glucan to ensure microbial clearance and reduce mortality rate of infected animal through its activities on macrophages and neutrophils, (Kaiser et al., 1993) and also that not much have been said on the intra-peritoneal administration of neutrophil without using it as an adjuvant or supplement in a particular formulation led into this study.

This study also tries to investigate the effectiveness of a new concentration on the protective effect of β- glucan administration through the peritoneum.

MATERIALS AND METHODS

Animals

Fourteen-weeks old mice weighing between 22 and 24 g obtained from the central animal house University of Ibadan were used in this study.

The mice were maintained at a maximum of 5 mice per cage under standard laboratory condition. The mice were fed with pellets obtained from Ladokun feeds in Ibadan and water was supplied to the animal ad libtum.

Bacteria

E. coli; A multi-resistant strain of *E. coli* obtained from the medical microbiology laboratory of Baptist Medical centre Ogbomosho Oyo State Nigeria was used for this study. Strains were grown in nutrient agar and later in eosin methylene blue agar which is selective for *E. coli* .These were then subculture in nutrient after which the stock culture was prepared by harvesting the cell suspension from nutrient agar in normal saline. The stock was diluted and use in the protection study.

β-glucan

Beta-glucan was purified from the cell wall of *Saccharomyces cerevisae* by Professor J.K. Oloke of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria and was made available for this study.

Protection study

To investigate the protective of the intra peritoneal administration of beta-glucan treatment 4 groups (n = 10) of mice were administered single intra-peritoneal dose of β glucan at 10 mg per 1000 mg body weight of mouse in 0.2 ml of saline. Saline was also administered to those that were used as control 24 h before the animals were challenged with 0.2 ml 0f the lethal dose (which is 0.2 ml 0f 4×10^{-2} cfu/ml of *E.coli)* of *E.coli by* intra-peritoneal administration.

The controls were not infected with *E. coli* but were only injected with 2 ml of saline solution.

Animals were observed twice daily for ten days within which the study last. The survival rate was recorded following the cause of infection and percentage survival was calculated using the ratio of the surviving animal each day to the total number of infected animals.

Microbial clearance

At the end of this study which is the 11th day following challenge with the lethal dose of *E.coli,* the surviving animal were humanely slaughtered, and the liver were harvested, weighed, macerated and homogenize in 20 ml nutrient broth in an aseptic condition. The liver homogenate were serially diluted and 0.1 ml of the serially diluted liver homogenate at a concentration of 0.2 ml of 1×10^{-7} cfu/ml of each homogenate was seeded into eosin methylene blue agar and incubated for 24 h at 25°C in other to evaluate the number of the colony forming units per gram liver tissue

RESULTS

A single dose of β-glucan, given intra- peritoneally increased the number of animals that survived by 40% from one (10%) in the control group to five in the intervention group (50%).Table 1 and Figure 1.

β-glucan was able to increase the survival rate in the intervention group because of its ability to stimulate the host innate immune response. The enhanced microbial killing as observed by significant reduction in the microbial bio-burden of the livers of the treated animal is shown in Table 2.

Statistical evaluation

Comparison of groups with regard to mortality was made by student's t test; comparisons of quantitative data were made with student's t test. The result of this study was found to be significant at a probability value of $p \leq 0.05$.

DISCUSSION

Previous studies have documented the nonspecific immune-modulatory properties of insoluble polysaccharides as zymosan (Riggi and DiLuzio, 1961; Williams et al., 1987; Reynolds et al., 1982; Cook et al., 1982 in animal test system). Also, Ondoker et al. (1992) were able to show the possibilities of using the solubilized form of beta glucan via percutaneous and trans thoracic administration and through intra muscular injection of the beta glucan to abrogate intra-abdominal sepsis.

The observation that yeast glucans could abrogate the deleterious effects of a variety of microbial pathogen prompted considerable interest in determining exactly how such compounds interact with the host immune system. The Moribund finding of a specific cell surface receptor for P-1, 3 glucans on macrophages and granulocytes suggested that the protective effects for the glucans are provided by such cells (Czop and Austen, 1985; Czop, 1986; Easson et al., 1990; Sherwood et al., 1987), possibly by cytokine production and release, improve phagocytic killing, or increased production of phagocytic cells. Because PGG glucans bind to cells with

Table 1. Percentage surviving mice following *E.coli* infection. These animals were observed for ten days and the percentage survival rate was recorded from the ratio of the mice that survive after infection to the total number of animal used.

Days	Percentage Survival			
	Infected animal with treatment (%)	Treated animal without infection (%)	Infected animal without treatment (%)	Animal without infection and treatment (%)
1	90	100	20	100
2	50	100	10	100
3	50	100	10	100
4	50	100	10	100
5	50	100	10	100
6	50	100	10	100
7	50	100	10	100
8	50	100	10	100
9	50	100	10	100
10	50	100	10	100

Table 2. Average microbial clearance rate in animal infected with *E.coli* the surviving animals were slaughtered and the liver harvested was homogenized in 20 ml nutrient broth to carry out colony counting in other to evaluate the microbial load in the liver (which is reported in colony forming unit).

Treatment	Cfu/g Liver
Infected Animal without Treatment	4.0×10^7
Infected Animal with Treatment	0.5×10^7

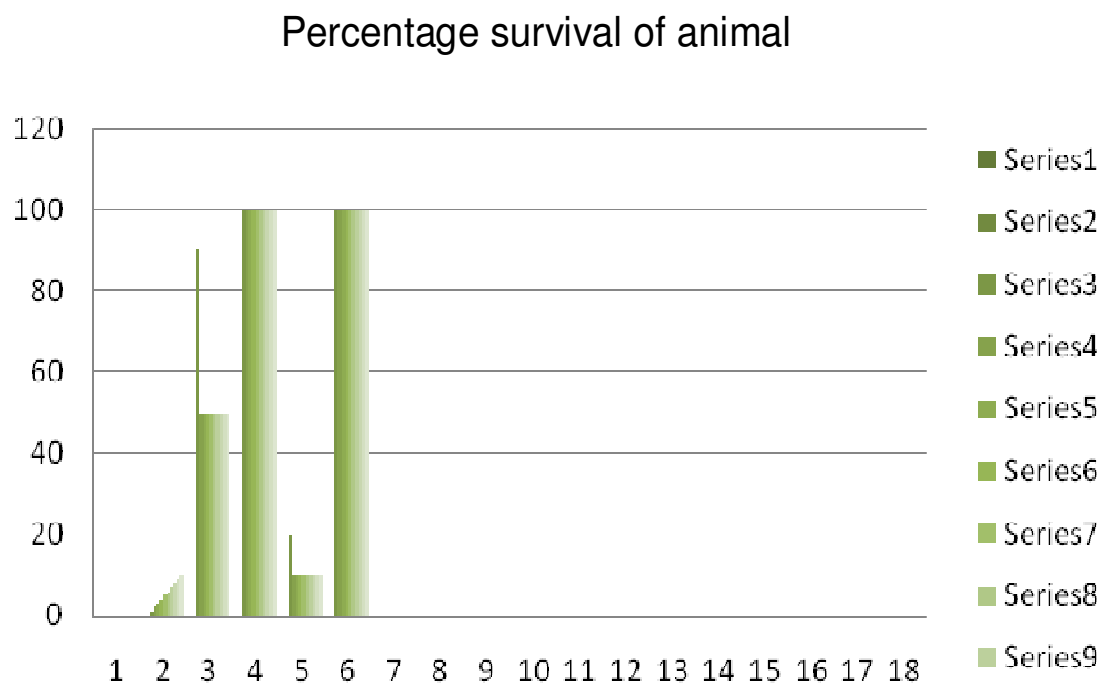

Figure 1. Histogram of the percentage rate of survival of animals after the completion of study. $p \leq 0.05$. Key 3 represent infected animal without treatment indicating how survival rate fell from 90 to 50%. 4 represent animals without infection but with treatment. 5 represents infected animal with treatment indicating how survival rate fell from 20 to 10% 6 rep animal without infection and without treatment.

an array of immune-modulatory activities, it is likely that the actual protection provoked by such materials is complex.

The protective effect of beta glucan is mediated through the stimulation of the microbicidal activities of the circulating blood cells of the innate immune system (Vetvicka et al., 1996).

The understanding of the ability of beta glucan to stimulate host immune defence and previous study that have been carried out on the efficacy of beta glucan prompted this study to investigate the protective effect of the intra peritoneal administration of beta glucan in an *in vivo* investigation.

The ability of beta glucan to significantly reduce the rate of death in infected animals as compare with other studies in which the effectiveness of beta glucan had been demonstrated in intramuscular administration further shows the ability of beta glucan to induce host immune cell in combating infectious diseases and like the result obtained by ondoker et al. (1992) soluble form beta glucan is also of significant important in combating microbial infection without any toxicity. The survival of the group of mice injected with beta glucan only further shows that the beta glucan in soluble form has no toxic effect and can be administered to animal directly instead of using it as an adjuvant.

Subsequent experiments had administered beta glucan to animals few hours before the introduction of infectious agents. This work however subject the animal to a 24 h treatment before exposure to infectious agents, during which there is possibilities of decrease in blood level of this protective agent. This therefore suggests two possibilities:

(1) This further establish the fact that the activities of beta glucan is based on its effect on the host immune system, and thus could possibly be a good agent in combating the continuous emergence of resistant disease condition.
(2) It also suggests the possibility of beta glucan to be used as a prophylactic agent against infection in the nearest future.

REFERENCES

Abass AK, Litchman AH, Pober JS (2008). Cellular and Molecular Immunology. 2nd edition Philadelphia, Pawb Sander Company,

Onderdonk ABR, Cisneros PH, Gary O. (1992). Anti-Infective Effect of Poly-13-6-Glucotriosyl-1,3-3-Glucopyranose Glucan *in vivo*. April 1992. J. Infect. Immun., 60(4): 1642-1647.

Bacon JSD, Farmer VC, Jones D, Taylor IF (1969). The glucan component of the cell wall of Baker's yeast (Saccharomycescerevisiae) considered in relation to its ultrastructure. Biochem. J., 114: 556-557.

Czop JK (1986). Phagocytosis of particulate activators of the alternative complement pathway: Effects of fibronectin. Adv. Immunol., 38: 361-398.

Czop JK, Austen KF(1985). A P-glucan inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement. Pathw. J. Immunol., 134: 2588-2593.

Czop JK, Austen KF (1985). Generation of leukotriene by human monocytes upon stimulation of their 13-glucan receptor during phagocytosis. Proc. Natl. Acad. Sci. USA, 82: 2751-2755.

Czop JK, Austen KF (1985). Properties of glucans that activate the human alternative complement pathway and interact with the human monocyte I-glucan receptor. J. Immunol., 135: 3388-3393.

Czop JK, Fearson DT, Austen KF (1978). Opsoninin dependent phagocytosis of activators of the alternative complement pathway by human monocytes. J. Immunol., 120: 11321138.

Easson DD, Ostroff Jr GR, James S (1990). Macrophage-targeted carbohydrate microcapsules for antigen and drug delivery. Abstr. Int. Congr. Infect. Dis., 58: 689.

Janusz MJ, Austen KF, Czop JK (1987). Lysosomal enzyme release from human monocytes by particulate activators is mediated by f-glucan inhibitable receptors. J. Immunol., 138: 3897-3901.

Kaizer AD, Kernodle BS (1998). Synergism between Poly- (1-6) beta – D- gluco- pyranosyl (1-3) – beta –d- glucopyranose.glucan and cefazolin in prophylaxis of *Staphylococcus infection* in a guinea pig model. Antimicrob. Agent Chemother., 42; 2449- 2451.

Kounnikasis B, Rose Monde MP, Brousseau G (2003). Anthrax protective effect of yeast beta, 1,3, glucan. MEDSCAP, Gen. Med., p. 5.

Phaff HJ (1963). Cell wall of yeasts. Annu. Rev. Microbiol., 17:15.

Riggi SJ, DiLuzio NR (1961). Identification of a reticuloendothelial stimulating agent in zymosan. Am. J. Physiol., 200: 297-300.

Sherwood ER, Williams DL, McNamee RB, Jones EL, Browdwe IW, DiLuzio NR (1987). Enhancement of interleukin-1 and interleukin-2 production of soluble glucan. Int. J. Immunopharmacol., 9: 261-267.