Effects of glucan on bone marrow

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Abstract: Bone marrow damage represents a significant problem in cancer treatment. Therefore, it is clear that the pharmacologic protection against bone marrow damage is of considerable interest, since the development of novel and effective medical approaches to combat radiation or cytotoxic damage are of major importance not only to the medical field but also to several industries and the military. This review represents a summary of our knowledge of the effects of various glucans on bone marrow protection.

Keywords: Glucan; mice; bone marrow; irradiation; chemotherapy

Introduction

β(1-3)(1-6)-D-glucans (glucan) represent a class of polysaccharides consisting of D-glucose monomers naturally found in fungi, algae, plants and bacteria, and are the major pathogen associated molecular patterns (PAMPs) of fungi, broadly recognized phylogenetically. Glucans have been studied extensively as an immune-stimulant in anti-infective, anti-tumor, immunoadjuvant in cancer therapy, wound healing, and for stress and the lowering of cholesterol (1,2). Readers seeking more information on glucan-related health benefits should read (3). Although many of the original studies with glucans were done via parenteral administration, several recent studies have demonstrated the possibility of oral administration without compromising glucan’s biological activities (4,5). Oral administration of glucan offers significant advantages such as easy administration, ease of mass distribution, and a relatively low cost. More importantly, orally administered glucans, both mushroom-derived (6,7) and barley-derived (8,9) have demonstrated their therapeutic efficacy.

Despite a long history of research, the exact mechanisms of glucan actions remain elusive. Numerous studies have indicated that yeast-derived glucans prime neutrophils and natural killer cells for action against iC3b-opsonized tumors as a result of complement activation by anti-tumor monoclonal or natural antibodies (10,11). With recent studies showing stimulation of humoral immunity including antibody response (12), it is clear that glucan-mediated immunotherapy may link both innate and adaptive immune responses.

Since glucan is nearing its use in clinical practice, attention has returned to the potential role of glucan implementing changes in the hematopoietic system. Effects of glucan on bone marrow and its cells is one of the first biological effects that have been described (13). From these studies, it was only a simple step to test the mechanisms by which glucans affect hematopoiesis in compromised (usually by irradiation or by chemotherapy) individuals. The model of irradiated animals was evaluated in 1980s, when Patchen and MacVittie began their study that showed glucan stimulated the bone marrow of irradiated mice (14). The effects of glucan on bone marrow after immunosuppression caused by chemotherapy were studied app. Ten years later when two different glucans were tested in a model of cyclophosphamide-induced suppression of bone marrow. The first data showed modestly faster restoration of bone marrow (15).
serious is the last one, because it is already evoked by the lowest radiation doses (16). Irradiation of the whole body results in hemopoietic stem cell depletion, which is accompanied by the loss of terminally differentiated leukocytes (17), lymphoid cells, and immunocompetent cells (18,19). Loss of immunocompetent cells and the decrease of their functional capability lead to severe opportunistic infections that, without adequate therapy, have fatal consequences (20,21).

The main strategy of treating hemopoietic syndrome consists of using so called biological modifiers, the substances of different origin and chemical composition, which have specific radioprotective properties. Radioprotective effects exert endotoxins and other bacterial derivatives (22,23). Animal studies suggest that Corynebacterium parvum and Bacillus Calmette-Guérin (23-26) have a protective effect in the stimulation of hemopoiesis and support of functional differentiation of immunocytes from their precursors. Increase of the concentrations of colony forming units (CFU) in bone marrow and spleen, as well as distinctly higher leucocyte and granulocyte numbers, were observed in the peripheral blood of treated animals.

In 1941, the so called zymosan, a rough fraction isolated from Saccharomyces cerevisiae cell wall, caused hyperplasia and hyperfunction of the reticuloendothelial system when administered to experimental animals (27). Later studies showed that the direct intravenous injection of zymosan could activate the immune system, stimulating protective host responses and stimulating phagocytosis in mice (28). Zymosan was also proven to stimulate hepatic erythropoietin production (29). S. cerevisiae cell wall consists of three layers: an inner layer containing insoluble glucan (30-35%), a middle layer of soluble glucan (20-22%), and an external layer composed of glycoprotein (30%). Zymosan also contains a variety of other components (mannans, chitin, proteins, and lipids) but the substances responsible for most of its observed biological effects, including stimulatory influence on the reticuloendothelial system (hemopoietic recovery) and immunity, is caused by glucan.

In the 70s-to-90s of the last century, glucans were shown to act as broad-spectrum enhancers of host defense mechanisms (30,31). This fundamental statement has been based on experimental results showing that the glucans optimized the immune response of experimental animals to infections of viral (32), bacterial (33,34), and fungal (35,36) origin (37-39).

Because the immune and hemopoietic systems are closely interconnected, the attention of researchers investigating effects of glucan on the immune response also focused on the consequences of its administration on hemopoiesis studying its influence on all differentiation stages from pluripotential stem cells through progenitor and precursor cells to mature effector peripheral blood cells.

During seventies, it was also convincingly proven that glucans are responsible for stimulation of mononuclear phagocyte system such as the bacterial substances mentioned above. It was hypothesized that that glucan administration leads to formation of some humoral factors that stimulate myeloid and erythroid proliferation (40,41). Others showed that glucan could reverse the myelosuppression produced with chemotherapeutic drugs (15). These authors have opened the way to the research aimed at testing glucan under conditions of hemopoietic suppression of various etiologies, especially after radiation- or chemotherapy.

Protective effects of glucans, both particulate and soluble, to irradiation and support of reparation after hemopoietic injury were convincingly demonstrated in vivo and in vitro by two research groups namely Patchen’s in USA (42-47) and Pospíšil’s in the Czech Republic (48-53). The first findings on support of hemopoiesis after irradiation by particulate glucans were reported by Patchen and MacVittie in 1982. In the same year, Pospíšil et al. published that a single administration of particulate glucan given previously or after whole body irradiation of mice by 60Co γ-rays enhances hemopoietic activity accompanied by increase of spleen weight, bone marrow cellularity, and peripheral white blood cells counts (48). In 1984, Patchen’s group published a report on modulation of hemopoiesis by nine different soluble polyglycans including several glucans applied before or after irradiation. It was shown that most of the soluble glucans had comparable stimulating effects on hemopoiesis comparable to particulate glucans. Patchen et al. (54) also reported that soluble glucan amended suppression of hemopoiesis by the quinolone antibiotic pefloxacin in irradiated mice.

In 1991, the Pospíšil’s research group also published results that soluble glucan given 24 hours before irradiation of mice with a sublethal or nearly lethal dose of γ-rays increased the number of bone marrow cells. In 1995, these authors confirmed the results by determining that soluble glucan, either in a single dose or repeatedly after exposure of mice to a sublethal irradiation, had positive effects on the recovery of compartments of progenitor and precursor cells in bone marrow.

Special attention was also devoted to the study of hemoprotective properties of glucan administered in combination regimens with some radioprotectants like...
selenium, cysteine and amifostine (WR-2721), which lack the toxicity associated with cysteine (55-58). The best radioprotection was reached by a combination of the above-mentioned substances. Enhanced restoration of hemopoiesis after irradiation was also observed with soluble glucan applied together with diclofenac, an inhibitor of prostaglandin production (59,60). The use of glucan in an ex vivo model increased the short-term colony forming capacity of human bone marrow mononuclear cells, which were influenced by granulocyte-macrophage colony stimulating factor (GM-CSF) (61).

It was also documented that orally administered water-soluble glucan from Lentinus lepideus every day for 24 days to irradiated mice (whole body irradiation) increased significantly the levels of IL-1β, IL-6, and granulocyte-macrophage colony units GM-CFU. On the other hand, the increased level of TNF-α due to irradiation was decreased. This means that glucan increased serum levels of radioprotective cytokines, while decreasing the level of radio-induced TNF-α the production of which was originally increased by tissue injury and anemia- caused radiation. In the bone marrow, the number of granulocytes and myeloid progenitors increased and further analysis of surface proteins in bone marrow cells indicated L. lepideus glucan might induce differentiation of progenitor cells to mature granulocyte population. In serum, the levels of GM-CSF, IL-6, and IL-1β were also substantially higher (62). Application of water soluble glucan extracted from L. lepideus to human peripheral blood mononuclear cells strongly increased production of TNF-α, IL-1β, IL-10, and IL-12 by two and three orders of magnitude but levels of GM-CSF and IL-18 were activated only by one order of magnitude. TNF-α was a first activated cytokine, which was detectable at 2 h after glucan treatment. Then the IL-1β followed after 6 h and IL-12 and IL-10 were next to follow. Contrarily, IFN-γ and IL-4 were not affected. GM-CSF and IL-18 increased after 24 h. Monocytes and macrophages were main cells type responding to glucan, but not T and B cells. Simultaneously, cellular transcription factor NF-κB was strongly activated (63). In another experiment it was demonstrated that soluble yeast-derived glucan could also enhance the proliferation of hematopoietic stem cells and support cellular recovery following sublethal irradiation, and increase the survival of lethally irradiated animals following allogeneic hemopoietic cells transplantation in a CR3-dependent manner. It suggests that during restoration processes the complement or some is components take part and that glucan is a ligand of the complement receptor 3 lectin-like domain and that support complement-mediated recovery after bone marrow injury (64).

**Glucan and chemotherapy**

Bone marrow hemopoietic suppression and decrease of blood cell populations represent major damaging consequences in anticancer chemotherapy. Due to the desperate need to find new antimutagenic agents and to determine their mechanism of action, chemopreventive efficiency, i.e., antimutagenic and antigenotoxic properties of glucans were studied both in vitro and in vivo.

Using the micronucleus assay in Chinese hamster ovary (CHO-k1) and hepatoma tissue cell (HTC) lines treated by mutagenic agents the methylmethane sulfonate (MMS) and 2-aminoanthracene (2AA), a mechanism of action of the glucan was studied. It was shown that glucan effectively protected CHO-k1 cells treated with MMS, whereas only the highest doses of glucan acted in prevention in HTC cell line influenced with the same mutagen. In the treatment of HTC cells, the effect of glucan was greatly diminished. The possible explanation would be that interaction between glucan and MMS and 2AA produced different metabolites, which could induce a different damaging effect on the cells. It is also possible that the mutagenic effect could be more pronounced in some protocols using glucans for antimutagenic therapy, due to its combination with different mutagenic substances (65,66).

Lin et al. (67) reported as the first authors that a fraction from Maitake mushroom, in which the active component is 1,6-glucan with β 1,3-branches, has a dose-response effect on mouse bone marrow hematopoeisis in vitro. The addition of glucan fraction significantly enhanced the development of CFU-GM colonies and promoted the recovery of CFU-GM colony formation after bone marrow cells were pretreated with doxorubicin.

In mice and cynomolgus monkeys treated with either myelospressive or myeloablative doses of cyclophosphamide, prophylactic administration of glucan was shown to accelerate the recovery of peripheral blood leukocytes, particularly neutrophils (47). Pretreatment mice with glucan substantially reduced the number of bone marrow and spermatigonial cells with chromosomal aberrations (68). Intraperitoneal, intravenous or oral administration of ultrasonically depolymerized carboxymethy lglucan prior to cyclophosphamide treatment of mice significantly decreased its clastogenic effect in bone marrow polychromatic erythrocytes. The protective effect was better with higher doses of glucan and even orally administered
glucan was sufficiently effective. The authors suggested that smaller molecules of depolymerized glucan easily passed through the gut wall into the circulation and were directly utilized within the bone marrow (69,70).

Oliveira et al. (71) also confirmed that glucan had a preventive effect on the clastogenic damage in pregnant and non-pregnant mice. It was documented that pregnant females were more susceptible to mutagenic damage. A trend toward a reduction in level of malformations was observed but teratogenic effects were not fully prevented. Even glucans did not prevent malformations, the increased fetal viability and enhancement of reproductive performance of females was documented. In further extensive study, the glucan isolated from *Saccharomyces cerevisiae* was applied in various amounts intraperitoneally to mice treated by cyclophosphamide (72). Mutagenous and genotoxic damage reduction was ascertained by means of micronucleus and comet assays. Within the first weekend of glucan treatment, the high genotoxic and mutagenic damage reduction was observed. Then, during sixth week of glucan application, the antimutagenicity rates dramatically decreased and antigenotoxicity was without any effects. The authors concluded that the effects of glucan to prevent damages of DNA may be limited after long time application and that the study of antimutagenic properties of glucan must be repeated to clarify mechanisms of its action.

**Conclusions**

The extensive research studying various effects of glucans on bone marrow showed significant restoration of both lymphopenia and neutropenia. These data led to a suggestion that glucan might be widely used as radioprotectant that could mitigate the biological effects of radiation exposure both in cases of radiation accidents or in medically used irradiation. With this knowledge, it was only a question of time before researchers determined that the same effects can be also found in bone marrow suppressed by cytotoxic drugs.

Taken together, the use of glucans gain another *Raison d’être*, as the therapeutic effects on bone marrow offered help for patients undergoing cancer treatment, either by irradiation or by chemotherapy. Despite the extensive research, only limited data are available on the mechanisms of glucan effects in irradiation or chemotherapy. However, as the bone marrow damage remains to be one of the most serious problems in cancer treatment, the pharmacologic protection against bone marrow damage is of considerable interest. From the data summarized above, it is clear that the glucans might offer an ideal solution—they are inexpensive, generally free from side effects and capable of significant protection against bone marrow damage through restoration of bone marrow cell production. At the same time, it is also clear that to progress further into clinical practice, more studies are needed.

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