Yeast Derived Beta-1,3-D-Glucan: An Adjuvant Concept

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According to the *Webster Medical Dictionary*, adjuvant (from Latin ad-juvo, to give aid to) is a substance added to a drug product formulation which affects the action of the active ingredient in a predictable way. This term has been widely utilized in immunology, where it means a vehicle used to enhance antigenicity of vaccines (for example, Freund's adjuvant). Much broader utilization of this term can be applied today to some naturally derived substances. This adjuvant concept closely relates to what is today referred to as a complementary/alternative modality.

Poly-branched beta-1,3-D-glucan is a naturally occurring polysaccharide that can be found in a variety of fungal cells including cell walls of yeast, *Saccharomyces cerevisiae.* As any other glucan (or polyglucose), it consists of glucose units linked together. For example, most starches are alpha-glucans. Out of different glucans, the beta-1,3-D-glucan configuration has been shown to act as a non-specific immune-activator.

Goldman, and later Czop, identified a specific receptor on the cells of macrophage origin that binds to the beta-1,3-D-glucan molecule. This receptor is a protein complex that appears to be present throughout the whole differentiation cycle of macrophages, starting in the bone marrow. Mature macrophages are found in virtually all the tissues including the central nervous system. When a macrophage encounters beta-1,3-D-glucan, it becomes activated. All the functions, including phagocytosis (ability to engulf foreign cells and particles), release of certain cytokines (intercellular hormones), and the processing of antigens are improved and brought up-to-date. Macrophages are extensively involved in everyday detoxifying processes, intestinal flora maintenance, anti-infective and anti-tumor protection and maintenance of overall health integrity. Although most of the research with this substance has been done in vitro and parenterally, later research at Baylor College of Medicine, sponsored by ImmuDyne, indicates the oral effectiveness of purified beta-1,3-D-glucan (Wyde, 1989).

The integrity of beta-1,3-D-glucan taken orally differs from other food substances. This type of glucan is acid resistant so it passes the stomach virtually unchanged. Further, in the intestine there is a lack of a specific enzyme (beta-1,3-glucanase) that would break it down to glucose or di-glucose so as to be absorbed through the intestinal wall. On the other hand, there are macrophages that inhabit the intestinal wall and are able to pick up beta-1,3-D-glucan particles through beta-glucan receptors. Immediate activation of these cells follows and later, they are able to travel back to the local lymph nodes (Payers Patches) as a part of their natural antigen-presenting function, to release cytokines (IL-1, IL-6, GM-CSF, Interferons) and induce systemic immune activation.

The mechanism described above is called phagocytic transport and it is common for certain microorganisms. Studies conducted with oral application of C13 labeled glucan also support existence of phagocytic transport for beta-1,3-D-glucan.

An adjuvant concept of pharmacological application for beta-1,3-D-glucan was suggested by DiLuzio in the 70s. This article is an attempt to overview this concept from today's perspectives utilizing modern knowledge of oral effectiveness, and a specific transport mechanism of beta-1,3-D-glucan.

There is now enough data to support the use of beta-1,3-D-glucan as an adjuvant in several important medicinal applications.

1. Combination "glucan + anti-infective agent"

Beta-1,3-D-glucan itself can elicit broad anti-infective effects. The nature of macrophage activation induced by this compound is non-specific. *Staphylococcus aureus, Eschericia coli, Candida albicans, Pneumocytis carinii, Listeria monococytogenesis, Leishmania donovani, Herpes simplex, Ascaris suum* - this is an incomplete list of microorganisms, against which a protective effect of glucan has been established. This list, as you can see, includes bacteria, fungi, viruses and parasites. None of the anti-infective agents possess such a broad spectrum of activity. Unlike an antibiotic compound interfering with metabolism of a pathogen, beta-1,3-D-glucan is a substance that modifies host response to cells genetically different from the host.

Numerous studies support the theory that an antibiotic and a macrophage activator work synergistically.

Experimental peritonitis in rats was used to show synergy between widely used antibiotic ampicillin and glucan. A 100% survival was the result of the combination treatment, while glucan alone gave 30% survival, and ampicillin in the given dose elicited 65% survival (20% survival in the control group). All the results were statistically significant (Lahnborg 1982).

A 56% survival was achieved when subtherapeutic doses of gentamycin was combined with intraperitoneally delivered glucan at just 0.1 mg/mice challenged with *Escherichia coli*. This was a very significant increase of survival rate, considering that either no treatment or this low dose of antibiotic alone, gave no protection from peritonitis (0% survival), while glucan alone gave 9% increase in survival. The difference between controls and the combination treatment was highly statistically significant (Browder, 1987).

Anti-fungal effect of beta-1,3-D-glucan from yeast cell wall is particularly interesting. It is known that glucan configuration in *Saccharomyces cerevisiae* resembles the one in *Candida albicans* . Glucan administered orally in mice with chronic generalized Candida infection, resulted in significant increase in the candidacidal activity of alveolar and spleen macrophages. The resistance not only to systemic infection with *Candida albicans*, but also *Staphylococcus aureus* increased, significantly reducing the growth of microorganisms in the kidneys of infected animals. Glucan also worked synergistically with the anti-fungal drug Amphotericin B (Nicoletti, 1992).

Although there is not enough data collected with regard to the anti-viral effect of glucan, there is now work in progress regarding its adjuvant anti-HIV effect.

Mortality, associated with *Herpes simplex* in mice was shown to be profoundly modified in early works and later, it was supported by oral studies (Wyde, 1990).

Mice treated with glucan both before and after the lethal viral hepatitis challenge, exhibited only limited liver pathology, minimal plasma enzyme alterations, and greatly enhanced survival versus a group receiving no treatment (DiLuzio, 1980). Macrophage phagocyting function, significantly impaired by hepatitis, was maintained by glucan application.

Another study shows that virally challenged mice have a limited wound-healing capacity that was corrected by systemic glucan application (Kenyon, 1983).

2. Combination "glucan + anti-neoplastic agent"

Glucan anti-tumor effect can be local or systemic. A local injection of beta-1,3-D-glucan suspension into melanoma lesions has been shown to successfully resolve the tumor locally (Mansell, 1978). In these human experiments, the site of injection revealed no previously established tumor, but plenty of macrophages filled with pigments. Obviously, macrophages were drawn to the site where they phagocytized and destroyed pigment-bearing tumor cells. These intralesional injections in some cases were able to control further growth of remote metastasis of the same tumor which resumed growth after glucan treatment withdrawal.

Unfortunately, no clinical applications were developed out of these results until recently (Carrow, 1996). The latest data contains very promising information, not only in regard to human melanoma, but also to basal cell carcinoma.

Experimental animal data on systemic applications of beta-1,3-D-glucan anti-tumor effects is abundant. Significant reduction in tumor growth and prolonged survival was observed in mice with transplanted melanoma. In mice with adenocarcinoma, there was an 85% reduction of tumor mass accompanied by prolonged survival. An anaplastic mammary carcinoma study showed results of 70% tumor regression and 80% survival versus 100% in the group with no glucan treatment. Chronic administration of glucan to aging mice with lymphocytic leukemia significantly improved their survival (DiLuzio, 1980). In these and other experimental models, systemic macrophage activation and certain cytokine releases, seem to be critical for clearing tissues from the tumor cells (Proctor, 1980) and inhibiting metastasis (Sakurai, 1991).

A combination of beta-1,3-D-glucan and an antineoplastic agent(s) might have a significant potential considering its a) direct anti-tumor effect, and b) ability to counteract chemotherapy-induced immunosuppression resulting in higher mortality from opportunistic infections.

The efficacy of glucan in combination with BCNU chemotherapy was measured using the disseminated transplantable leukemia; the combination yielded a high level (56%) of cures compared to no survival for either agent alone (Stewart, 1978).

Glucan application can also protect a patient from leukocytopenia (decrease in the number of peripheral leukocytes) associated with a chemotherapeutic agent, which is one of the major obstacles in the chemotherapy of cancer. A decrease in the number of peripheral leukocytes by 5-fluorouracil was prevented by the oral application of glucan in mice. Proliferative responses of bone marrow cells to granulocyte/macrophage colony stimulating factor (GM-CSF) or granulocyte colony stimulating factor (G-CSF) were suppressed by 5- fluorouracil treatment, and their recoveries were enhanced by glucan and serum level of cytokines such as IL-1 and IL-6 were increased (Miyazaki, 1992).

Interestingly, that use of corticosteroid hormones, also having immunosuppressive effect, and widely used as a part of chemotherapy programs or in autoimmune situations, might be another indication for use of beta-1,3-D-glucan in combination with this class of drugs. Goldman showed that the amount of beta-1,3-D-glucan binding capacity of macrophages increases when they are exposed to hydrocortisone. She states that this might be a result of enhanced expression of beta-glucan receptor. A logical interpretation of that can be that it's an attempt to compensate the diminished phagocytic ability of macrophages exposed to this class of hormones.

3. Combination " glucan + radiotherapy”

This combination seems to be very logical in the light of the data mentioned above. Radioprotective (bone marrow protective effect) of yeast glucan is well-established and documented with the mechanism of enhancing hemopoietic recovery and hence, by regenerating the host's ability to resist life-threatening opportunistic infections. However, it also has been demonstrated that host resistance to opportunistic infection in glucan-treated irradiated animals is enhanced even prior to the detection of significant hemopoietic regeneration. This early enhanced resistance to microbial invasion could be correlated with enhanced and/or prolonged macrophage (but not granulocyte) function.

These results suggest that early post-irradiation glucan may mediate its radioprotection by enhancing resistance to microbial invasion mechanisms not necessarily predicated on hemopoietic recovery.

Experimental data suggest that glucan can also function as an effective free-radical scavenger (primarily toward hydroxyl radical). Because macrophages have been shown to selectively phagocytize and sequester glucan, it is possible that these specific cells may be protected by virtue of glucan's free-radical scavenging ability (Patchen, 1987).

Oral application of yeast beta-1,3-D-glucan for 20 consecutive days after a single, near lethal, dose of radiation resulted in 70-90% survival versus 30% in the control group.

4. Combination "glucan + topical agent"

Glucan is an excellent wound healer. In experiments glucan-treated wounds showed a higher number of macrophages in the early, inflammatory stage of repair, with fewer polymorphonuclear neutrophilic leukocytes than did control wounds. Both re-epithelization and the onset of fibroplasia commenced at an earlier stage in glucan-treated wounds than in control wounds. Five days following the incision, glucan-treated wounds were generally completely re-epithelialized, while control wounds were not. The organization of fibroblasts in glucan-treated wounds was more advanced at 5 and 7 days following injury, and the extent of fibroplasia was also greater. By 10 days following injury, glucan-treated wounds were completely re-epithelialized and no formation of granulomas was observed up to one month following wounding (Leibovich, 1980).

In humans, topical glucan treatment resulted in 73% improvement in chronic decubitus ulcers with complete closure and epitalization in 27% of treated ulcers. All wounds remained clean with no infections occurring during this treatment (DiLuzio, 1984).

Considering the data above, a topical combination of an antibiotic and beta-1,3-D-glucan as an adjuvant for wound healing applications, seems to be appropriate.

An interesting effect of topical application of glucan was observed in regard to non-wounded aged skin. Revitalizing, such as reducing the number, depth and length of wrinkles, thickening, reducing roughness and dryness of the skin was shown in a group of female volunteers (Smith, 1991).

Applied topically, glucan activates epidermal macrophages (Langerhans cells). This mechanism plus its free-radical scavenging effect makes it a photoprotective agent. Glucan application resulted in the reduction of after-UV erythema and preservation of the amount of Langerhans cells in the epidermis (Elmets, 1992). A combination of a sunscreen + glucan is suggested.

Anti-irritant effect of beta-1,3-D-glucan was also shown in combination with otherwise severe irritation causing levels of lactic acid (Smith, 1991). Glucan also has a synergistic effect with another anti-aging topical ingredient: retinoic acid (Retin-A). Similar to corticosteroids, Retin-A significantly increases the number of beta-glucan receptor-sites on phagocytic cells.

5. Combination "glucan + nutrients"

Very recent discoveries have been made on combined use of glucan and vitamin C derivatives.

Intracellular ascorbate content in phagocyting cells reaches 40 times the level of plasma ascorbates. Macrophages activated with beta-1,3-D-glucan exhibit a significant drop in the intracellular ascorbate content. This might lead to the exhaustion of free-radical scavenging capacity of these cells, as well as to impaired motility and certain enzyme production by macrophages.

There are products on the market now that combine beta-1,3-D-glucan and vitamin C derivatives to replenish ascobate levels in the glucan-activated macrophages. This is not only physiological from the standpoint of glucan pharmacological effects, but it also seems to have a great impact on results of Vitamin C treatments.

Commercial application of yeast derived purified beta-1,3-D-glucan, available in a dietary supplement form and in a pure form for compounding, started in 1995. There is obviously a lack of recent double-blind human studies but plenty of anecdotal clinical data ranging from tumor mass rejection to healing of chronic wounds. Hopefully, we will see more studies with beta-1,3-D-glucan in the near future as this substance gains acceptance within the medical community.

Clinical directions presented in this paper are not by any means a complete list of all possible applications and adjuvancy combinations with this substance. I believe that a thinking physician can find more ways to utilize this material in practice. Now, when we have a better understanding of its mode of action, we can prognose and prove in practice the benefits of using beta-1,3-D-Glucan by itself or by adding it to either conventional or alternative types of therapies that would affect such therapies in a predictable way, which in turn is a concept of adjuvancy.

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