Acemannan Review

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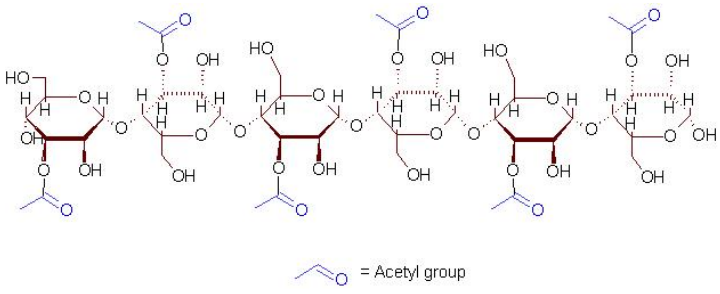
Chemistry and Manufacture

Acemannan, a β-(1,4)-linked polydispersed, highly acetylated mannan with an average molecular weight of approximately 1000kDa is obtained from the inner leaf of *Aloe vera* L., also commonly referred to as *Aloe vera* Barbadensis (Turner et al., 2004; (Tai-Nin Chow et al., 2005). The structure of the acemannan monomer is shown below.



Aloe vera gel is separated from the rind of the plant by filleting an aloe leaf and separating the inner gel from the outer leaf rind and the sap contained within the rind. The inner gel is then homogenized, extracted with alcohol, and further processed to yield a polydispersed high molecular weight complex carbohydrate. This carbohydrate was given the United States Adopted Name acemannan. The most common form of the product that has been used commercially consists primarily of acemannan, but includes other structural components of the inner leaf pulp such as galactose and galacturonic acid (Ni et al., 2004).

Assays for acemannan have been developed using size exclusion chromatography (SEC) or nuclear magnetic resonance (NMR) analytical methods (Turner et al., 2004; Davis & Goux, 2009).

Laboratory Studies

In vitro Immunomodulation Studies

Acemannan has demonstrated immunomodulatory effects in *in vitro* studies. In a study utilizing the mouse macrophage cell line RAW 264.7, nitric oxide and pro-inflammatory cytokines IL-6 and TNFα were released in a dose dependent manner when exposed to acemannan in combination with IFNγ (Zhang & Tizard, 1996). In addition, it was demonstrated that nitric oxide (NO) release resulted in apoptosis or programmed cell death of the RAW 264.7 macrophage cells. In a separate study, NO release was also demonstrated when chicken macrophages were exposed to acemannan (Karaca et al.,

1995). This effect potentially could contribute to some of the antitumor and antimicrobial effects that have been observed with use of the compound (Ramamoorthy & Tizard, 1998).

Whole Aloe vera gel, which contains acemannan and other components, has been shown to down regulate and suppress TNFα release from a human macrophage cell line (Habeeb et al., 2007). Thus it is evident that multiple components with immunomodulatory activity are present in the inner gel of the plant.

Further evidence of the immunomodulatory effects of acemannan were reported in a study showing that a 99% pure carbohydrate fraction of Aloe vera gel (i.e., acemannan) increased release of hematopoetic cytokines and stem cell factors (Talmadge et al., 2004). In addition, investigators have reported on the functional maturation of dendritic cells (antigen processing cells highly associated with the skin, mucosa of the lungs and GI tract) when exposed to the compound (Lee et al., 2001).

In addition to effect of acemannan on macrophage and dendritic cells, it has been shown to upregulate both function and generation of cytotoxic T-lymphocytes in a mixed lymphocyte culture (Womble & Helderman, 1992). Acemannan thus exhibits multiple immunomodulatory effects which are important to the healing of wounds.

In vitro Antimicrobial Activity

A review of the literature has shown studies related to the antimicrobial effects of acemannan. An *in vitro* comparative study was conducted to evaluate activity of various materials against *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli* and *Streptococcus agaiatiae* (Group B *Streptococcus*). Results indicated that the material containing acemannan with no other antimicrobial agents had limited effect on *E. coli and S. aureus* and no direct effect against *Candida* and Group B *Streptococcus* (Bidra et al., 2011). However, upregulation of macrophages by exposure to acemannan demonstrated increased phagocytosis and killing of *Candida albicans* (Stuart et al., 1997).

An *in vitro* study with a pneumocyte cell line was conducted to determine if acemannan had an effect on the binding of *Pseudomonas aeruginosa*. The study was based on the interaction of Gram-negative bacteria with host cells through host cell surface glycoconjugates, and it was thereby postulated that acemannan could block the adherence of the organism. Results demonstrated that acemannan did significantly reduce adherence of *P. aeruginosa* in a concentration dependent manner. However, acemannan also continued to interfere with binding following exposure of the cells to Con A, thereby indicating that the mechanism for blockage is probably not completely associated with mannose-containing cellular receptors (Azghani et al., 1995).

Acemannan has also been shown to have *in vitro* activity against HIV-1 with the effect attributed to modification of the virus-mediated glycosylation process (Kahlon et al., 1991). A series of *in vitro* experiments were conducted to evaluate the synergistic antiviral effects of acemannan in combination with azidothymidine (AZT) and acyclovir (ACY). T4-

lymphocyte and Vero cell lines were used as host cells for HIV-1 and Herpes simplex virus type 1 (HSV-1) respectively. Results indicated that acemannan possessed a dose dependent synergistic effect with AZT on HIV-infected cells. Relating to HSV-1, acemannan alone had approximately a 22% reduction of the cytopathic effects of the virus; however, when combined with ACY at optimal drug concentrations, the cytopathic effect was reduced by greater than 90% (Kahlon et al., 1991).

In vivo Wound Healing

Acemannan administered by different routes has been shown to impact healing. The effects of acemannan were evaluated in a number of *in vitro* and *in vivo* studies. *In vitro* cytokine and collagen release from gingival fibroblasts (GF) prepared from explanted culture of tissues obtained through surgical removal of impactions was determined following exposure to acemannan. In addition, an *in vivo* wound healing assay was conducted using Sprague Dawley rats to assess the impact on healing by acemannan compared to normal saline as the negative control, with triamcinolone dental paste and a Carbopol® gel as positive controls. Results of the *in vitro* studies demonstrated GF proliferation as well as significantly increased secretion of keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF) and Type I Collagen in acemannan-treated GF cells. The *in vivo* study showed that the Carbopol gel containing 0.5% acemannan significantly impacted healing as compared with triamcinolone, Carbopol with no additives, and Carbopol containing either 1% or 2% acemannan (Jettanacheawchankit & Sasithanasate, 2009).

A separate set of studies was conducted involving an *in vitro* evaluation of primary human dental pulp cells treated with acemannan. An *in vivo* study was conducted by the same investigators to evaluate the effect of acemannan on healing of surgically exposed first molars of Sprague Dawley rats. Acemannan significantly increased pulp cell proliferation as well as stimulated dental pulp healing (Jittapiromsak et al., 2010).

In a much different use of an acemannan containing wound gel, the product was evaluated topically in a series of experiments on C3H mice receiving graded single doses of gamma irradiation to the right rear leg creating an acute radiation-induced skin reaction. The peak skin reactions of the acemannan treated mice was reduced by as much as two fold as compared to controls when product was applied immediately following irradiation and continued daily (Roberts & Travis, 1995).

Non-Clinical Toxicology

The Cosmetic Ingredient Review Expert Panel conducted a safety assessment review of Aloe vera leaf materials. The review of acemannan had the following findings (Cosmetic Ingredient Review Expert Panel, 2007):

- 14 Day NOEL (no observable effect level) in the diet of Sprague Dawley rats was 4.1-4.6 g/kg/day

- No significant toxicity was seen in mice, rats or dogs at maximum dose levels of 200, 50 and 50 mg/kg respectively, administered ip or iv at 4 day intervals over 30 days Doses of 1500 mg/kg/day in the diet of dogs and doses up to 2000 mg/kg/day in the diet of mice for 180 days had no observable effect

The studies summarized above are further described by Fogleman et al. (1992).

Clinical Wound Healing Studies

Veterinary

A number of small studies in companion animals have been conducted with topical use of acemannan incorporated in a wound gel dressing. A review of its indications in treatment of second intention wounds is discussed with its usefulness being described as best in the first seven days of healing (Dart et al., 2005). Results have been limited primarily to case reports.

Human

Most of the human data for topical use of acemannan has been case based and is not available to the author. Two studies have been published. The first relates to use of an acemannan hydrogel dressing compared to saline for the treatment of pressure ulcers. Thirty patients were included in the study and randomized either to the acemannan group or saline group. Application of either dressing was done on a daily basis for the duration of the study. No significant difference was seen between the two groups (Thomas et al., 1998). Approximately half of the ulcers treated were Stage II and it is generally reported that such ulcers heal without complication. Due to the small sample size it is difficult to draw any meaningful conclusions from this particular study.

A freeze dried patch containing acemannan (SaliCept) was retrospectively compared to a clindamycin-soaked GelFoam patch applied post surgically to mandibular third molar extractions to evaluate the impact of each treatment on the incidence of alveolar osteitis (AO; also commonly known as dry socket). Evaluation was retrospective and not concurrent. All patients on study were treated with the SaliCept patch for a 2-year period with the results compared to all patients previously treated for a 2-year period with GelFoam soaked in clindamycin. All records were examined in collection of data. There

were 1,194 patients in the study of which 587 were in the GelFoam group and 607 were in the SaliCept patch group. Analysis showed that a significantly greater number of patients in the GelFoam group, 8% as compared to 1.1% for the SaliCept group, developed AO. One interesting factor revealed by the study related to the impact of smoking on the incidence of AO in the GelFoam treatment group. Smokers had almost twice the incidence of AO (13.3% vs. 7.0%) compared to non-smokers. However, none of the smokers treated with the acemannan patch developed AO (Poor et al., 2002).

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