ANTI-INFLAMMATORY AND IMMUNOMODULATING ACTIVITY OF GALLIUM

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Gallium has shown anti-inflammatory and immunomodulating activity in some in vitro and animal models of autoimmune disease, inflammatory disease, and allograft rejection. The data suggest that clinical testing of gallium may be warranted for (1) treating autoimmune diseases, such as rheumatoid arthritis, psoriasis, lupus, and multiple sclerosis; (2) treating some inflammatory diseases, such as asthma; and (3) ameliorating acute rejection following organ transplantation. Much of the following report is taken from the review by Bernstein (1998), updated to November 2008.

The ability of gallium to affect immune responses was first noted by Maurel (1973) and Bouissou et al. (1973). They found that a low dose of gallium (0.46 mg Ga/Kg/day as subcutaneous gallium sulfate for five weeks) aggravated the progression of tuberculosis in guinea pigs and suppressed the allergic response in infected animals to tuberculin injections. The authors hypothesized that gallium was suppressing macrophage and/or T-cell response. Delbarre and Rabaud (1976) found that gallium (0.74 and 1.48 mg Ga/Kg/day as gallium sulfate administered subcutaneously for 19 days) suppressed the cutaneous tuberculin response in rats. It is noted, however, that some more recent studies (Larry Schlesinger et al., unpublished data, 1999) found that gallium (at 0.51, 1.6, and 4.7 mg Ga/Kg/day for 28 days as orally administered gallium maltolate) did not have these effects, but actually inhibited infection by Mycobacterium tuberculosis in guinea pigs.

Several other animal and in vitro studies have shown gallium to suppress certain immune reactions, without being generally immunosuppressive or cytotoxic. Gallium appears to target specific inflammatory and proliferative responses, particularly those mediated by T-lymphocytes and macrophages. A few representative studies are briefly discussed below.

Effects of gallium on T-cells and T-cell mediated autoimmune disease

Gallium sulfate (daily doses of 0.185 - 1.48 mg Ga/Kg from day 0 of study, or 1.48 mg Ga/Kg on day 3; Delbarre and Rabaud, 1976) or gallium nitrate (30 mg Ga/Kg on day -1, then 10 mg Ga/Kg weekly; Matkovic et al. 1991) administered subcutaneously to rats suppressed the development of adjuvant arthritis, a T-cell-mediated autoimmune disease with similarities to Reiter’s disease and rheumatoid arthritis in humans. Animals that received gallium developed less synovitis, pannus, subchondral resorption, cartilage degeneration, and periosteal new bone formation than control diseased animals. Related in vitro studies on purified-protein-derivative-specific rat T-cells found gallium to block both antigen-specific and mitogenic proliferative responses (Matkovic et al., 1991). Interestingly, the same cell line exposed to gallium but not to antigen or mitogen showed normal to slightly enhanced proliferative activity, indicating that gallium was not directly toxic to the cells. Delbarre and Rabaud (1976) found that lymphocytes from gallium treated rats had normal responses to specific and non-specific mitogens.
Experimental autoimmune encephalomyelitis (EAE), a T-cell mediated autoimmune disease used as a model for demyelinating human diseases such as multiple sclerosis, was also suppressed in rats by weekly subcutaneous administration of gallium nitrate (Whitacre et al., 1992). When only a single gallium injection was administered, the timing was important: maximum disease suppression occurred when administration was on day 6 following induction of EAE, with less suppression resulting from administration on days 3 or 9, and none on day 12. Lymphocytes extracted from gallium-treated and untreated rats were tested *in vitro* for their proliferative responses. The proliferative response to myelin basic protein (MBP; the antigen used to induce EAE) was suppressed in cells from gallium-treated animals (treated on days 3, 6, 9, or 12) compared to those from non-treated animals. The proliferative response to the mitogen concanavalin A (Con A), however, was not suppressed in cells from gallium-treated animals, indicating that gallium did not have a general toxic effect. Further experiments, using a separate MBP-specific T-lymphocyte line, found that the proliferative response of such cells to MBP was suppressed when gallium was added to the cell cultures within 48 hours of initiation of culture; no effect was seen at 62 hours. All these results indicate that gallium acts to suppress T-cell proliferation at early stages of activation, and is not simply toxic to T-cells.

Similarly, gallium nitrate (30 mg Ga/Kg at -1 day; 10 mg Ga/Kg at 1, 4, 7, 10, 13, 16, and 19 days) significantly inhibited the development of experimental autoimmune uveitis in rats (a T-cell mediated disease, induced by injecting the rats with a solution containing retinal S-antigen and *Mycobacterium tuberculosis*) (Lobanoff et al., 1997). Gallium was highly effective at preventing clinical and histological signs of retinal and choroidal inflammation. Lymphocyte proliferative responses in Ga-treated rats to S-antigen, purified protein derivative, and Con A were all decreased by small though significant amounts. Gallium also caused a small decrease in the humoral immune response, measured by a reduction in antibody production to S-antigen.

In a mouse model of systemic lupus erythematosus (MRL/Mp lpr/lpr mice, which spontaneously develop a lymphoproliferative disorder characterized by the development of autoantibodies, DNA-antiDNA complexes, and autoimmunologic damage to the kidneys, joints, lungs, skin, and other tissues and organs), gallium nitrate suppressed progression of the disease (Apseloff et al., 1997). Mice that received a subcutaneous injection of 45 mg Ga/Kg at three weeks of age, followed by weekly injections of 15 mg Ga/Kg for 12 weeks, had significantly reduced glomerulitis, renal vasculitis, and lymphoid infiltrates in the lungs, spleen, and lymph nodes compared to vehicle-treated mice.

Gallium may have moderate efficacy in ameliorating asthma in B6D2F1/J mice (Apseloff et al., 1996). Asthma was induced in mice by subcutaneous injection of ovalbumin (OVA) on days 0 and 5 of the experiment, followed by exposure to aerosolized OVA on day 12; the mice were euthanized on day 14. Mice that received 45 mg Ga/Kg as gallium nitrate on day 11 showed a significant reduction in histological evidence of asthma compared to mice exposed to OVA but only given saline on day 11. A subsequent experiment in which a small group of mice was given 45 mg Ga/Kg of gallium nitrate on days 6 and 11 failed, however, to show efficacy.

In a model of endotoxic shock, gallium nitrate (45 mg Ga/Kg, subcutaneous injection) administered 24 hours prior to lipopolysaccharide (LPS) injection in *P. acnes* sensitized mice attenuated LPS-induced hepatitis but had no effect on production of tumor necrosis factor-alpha (TNF-α) (Krecic et al., 1995). Further studies of this model (Krecic-Shepard et al., 1999) found
that gallium nitrate reduced liver damage (lowered inflammatory infiltrates, hepatocellular injury, and necrosis) and suppressed the production of nitric oxide, but again had no effect on TNF-α production.

In a mouse model of Type I diabetes, gallium suppressed the development of diabetes in non-obese diabetic mice (Flynn et al., 1992). In one study, subcutaneous gallium nitrate at a dose of 45 mg Ga/Kg was administered at six weeks of age, and then weekly doses of 15 mg Ga/Kg were administered until 20 weeks of age. At 30 weeks, no treated animals had developed diabetes, whereas all of the control animals were diabetic. Although some diabetogenic T-cells were still present in the treated animals, their activity was greatly curtailed.

Several other studies have confirmed that gallium is a potent inhibitor of T-cell activation and proliferation in rodents and in vitro. Drobyski et al. (1996) found that transferrin-gallium (Ga-TF) markedly suppressed alloantigen-induced proliferation of mixed lymphocytes; it also significantly reduced the density of IL-2 receptor on activated T-cells and slightly reduced the number of CD3+/CD25+ T-cells in phytohemagglutinin-stimulated cultures. Similar to the iron chelator deferoxamine, Ga-TF significantly increased the density of transferrin receptor (CD71) and the level of transferrin receptor mRNA in activated T-cells, but did not affect the number of these cells. Importantly, Ga-TF did not inhibit IL-2 secretion or the induction of IL-2-stimulated lymphokine-activated killer activity. Huang et al. (1994) found that while gallium suppressed T-cell activation and some lymphokine [including interferon-gamma (IFN-γ)] secretion in cell cultures, it did not directly interfere with the normal inflammatory response of gonadal vein endothelial cells (GVEC), including their response to IFN-γ and TNF-α. This GVEC response, which includes the production of intercellular adhesion molecule-1, favors tissue growth and repair, and actually appeared to be enhanced by gallium.

These experimental results regarding T-cells suggest that gallium in the animal models investigated suppresses abnormal T-cell activation and proliferation, while having little effect on normal immune and inflammatory responses. Some of the selective anti-inflammatory activity may be due to pro-inflammatory T-helper type 1 (Th-1) cells being much more sensitive to inactivation by iron deprivation than anti-inflammatory, pro-antibody Th-2 cells (Thorson et al., 1991). Gallium is thus expected to be anti-inflammatory without being generally immunosuppressive.

**Effects of gallium on macrophages**

The immunologic effects of gallium on macrophages have also received some attention. Gallium was found to transiently inhibit the expression of major histocompatibility complex (MHC) class II by murine macrophages (Matkovic et al., 1991). In activated murine macrophage-like RAW 264 cells, gallium was found to inhibit dose-dependently the secretion of IL-6, TNF-α, and nitric oxide (NO) (Makkonen et al., 1995). Mullet et al. (1995) found that gallium nitrate inhibited NO secretion from activated murine ANA-1 macrophages, but did not inhibit secretion of TNF-α.
Effects of gallium on allograft rejection

The selective immunosuppressive properties of gallium have led to its testing as a possible anti-rejection therapeutic for allograft subjects. In a mouse model of severe graft versus host disease (utilizing irradiated that received transplanted bone marrow and spleen cells), citrate-buffered gallium nitrate administered by continuous infusion over 14 days at 2.6, 3.5, and 4.6 mg/Kg/day significantly prolonged survival and attenuated effects of the disease (Drobyski et al., 1996). In another study (Orosz et al., 1996), survival was greatly extended and histological evidence of tissue rejection was reduced in C57BL/6 mice that received cardiac allografts and were treated with gallium (as subcutaneous citrate-buffered gallium nitrate, 30 mg/Kg on the day of transplantation and on the third day post-transplant, then 10 mg/Kg every third day until day 30). Although acute rejection was inhibited, chronic rejection, including persistent inflammation, was observed (Orosz et al., 1997). Gallium was not found to affect the inflammatory immunological response to skin allografts (Sirak et al., 1997). In addition, gallium did not affect acute cardiac allograft rejection in Balb/c mice or in a primate model (Charles Orosz, oral communication, 2002).

Hypotheses on further mechanisms of immunomodulating activity

Ghio et al. (1997) hypothesize that neutrophilic inflammatory responses (which include the secretion of large amounts of lactoferrin) are triggered in part by iron chelates and chelators that are not indigenous to the organism (including bacterial siderophores). Their hypothesis includes the postulate that Fe$_{3+}$ is reduced to Fe$_{2+}$ in an attempt to free the iron from its exogenous chelate, through superoxide generated by phagocyte associated NADPH oxidoreductase in the neutrophil; the Fe$_{2+}$ is then reoxidized and combined with lactoferrin. It is tempting to speculate that gallium, which can act as a ferric analog but cannot be physiologically reduced, and perhaps differing biochemical behaviors of gallium-lactoferrin and iron-lactoferrin following uptake by activated macrophages (and possibly T-cells), may act to suppress the inflammatory response.

As some autoimmune diseases may actually be caused by infectious organisms, it is also possible that gallium may exert therapeutic activity through its antimicrobial properties. For example, rheumatoid arthritis has been hypothesized to be caused by mycoplasmal or bacterial infection (e.g., Horowitz et al., 2000; Johnson et al., 2000), and infection is strongly implicated in the etiology of multiple sclerosis (e.g., Steiner et al., 2001).

In addition to the immunomodulating mechanisms discussed above, gallium may inhibit some immune reactions by antimitotic activity on certain lymphocytes. It is, in fact, possible that this will turn out to be the dominant mechanism.

Experimental data on oral gallium maltolate in rat models of inflammatory arthritis

To study the anti-inflammatory effects of gallium maltolate, the compound was administered orally to rats that had induced inflammatory conditions. The two conditions examined were adjuvant induced arthritis (a very severe, acute, generally fatal inflammatory condition that causes joint inflammation, bone degradation, plus swelling and damage to the liver
and spleen) and streptococcus cell wall induced chronic arthritis (a more chronic condition characterized by joint and bone inflammation and degradation). In both conditions, oral gallium maltolate reduced joint inflammation, bone degradation, liver and spleen enlargement, and other measures of inflammation in a dose-dependent manner. In some measures of activity, gallium maltolate was more effective than the steroid dexamethasone or the immunosuppressant cyclosporine (Figures 1, 2). These experiments were carried out under the supervision of Dr. Alison Bendele at the University of Colorado at Boulder. (Also see Schwender et al., 2005.)

Figure 1. Effects of oral gallium maltolate at 100 and 300 mg/Kg/day and of oral dexamethasone at 0.1 mg/Kg/day for 21 days on liver enlargement in rats with adjuvant induced arthritis.

Figure 2. Effects of oral gallium maltolate at 100, 200, and 300 mg/Kg/day and of oral cyclosporine at 5 mg/Kg/day for 28 days on periosteal proliferation in rats with streptococcus cell wall induced chronic arthritis.
References Cited


