Anhang/Appendix U

# A REVIEW OF THE MODULATION OF RADIATION EFFECTS BY EICOSANOIDS AND CYTOKINES

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## INTRODUCTION:

Eicosanoids have relatively simple chemical structures which belie their extremely complex interactions and effects on cells, tissues, and whole organisms. The cytokines are a much more diverse and chemically complex family of compounds; however, they too have a large number of cellular actions. Among the vast array of documented effects of these classes of hormone-like naturally occurring compounds, both contain some compounds that have been found to modulate radiation injury. The influence of eicosanoids and cytokines on radiation injury is both specific to each agent and to each tissue within the organism. Protection or sensitization of cells to radiation may be locally or regionally modulated through direct or indirect means; however, it is likely that most of the effects of both eicosanoids and cytokines are indirect through the induction of specific biochemical pathways and events. These compounds may exert their influence on radiation injury by altering the cell cycle distribution within tissues, stimulating cell proliferation and accelerating recovery of tissue systems, affecting DNA repair pathways, influencing second messenger pathways, altering electrolyte balances and a variety of other physiological effects. In addition, it is well known that both eicosanoids and cytokines are produced in response to radiation injury and they are likely to be involved in what has become known as a bystander effect in which effects can be seen on unirradiated cells adjacent to, or at

some distance from cells that have been hit by a photon or by particulate radiation (1). Since these compounds are associated with nearly all physiological and pathological events in mammals, sorting out true cause-effect relationships is a daunting task. Although the exact mechanisms of how eicosanoids and cytokines influence radiation injury are not well known to date, progress toward understanding these mechanisms is inextricably woven into a better understanding of the biology and physiology of cells and tissues and of mammalian systems in general. The intent of this review is to briefly summerize the available data and provide some insight into the role of these compounds in radiation injury and to provide a view of how these agents may be used in the future for the prophylaxis and/or treatment of radiation injury.

## EICOSANOIDS: PRODUCTS OF THE ARACHIDONIC CASCADE:

A potent smooth muscle contracting component of secretions from seminal vesicles and the prostate gland was recognized in work done in the mid 1930s by Goldblatt and von Euler (14, 72). Because of the origin of these secretions, the unknown bioactive agent(s) were called vesiglandins or prostaglandins (PGs). Currently, the preferred designation for these bioactive products of the arachidonic acid cascade is eicosanoids; a term derived from their chemical structures; however, the term PGs is used extensively in the literature. In the late 1950s, Bergstrom (4) developed techniques to isolate and characterize eicosanoids and Samuelsson (60) developed techniques to synthesize PGs in the 70s. Eicosanoids have now been identified in most tissues of most members of the animal kingdom (64).

Endogenous eicosanoids are products of two major pathways mainly associated with the metabolism of membrane stored arachidonic acid (AA); the cyclooxygenase (COX) pathway

leading to PGs and the lipoxygenase (LOX) pathway leading to leukotrienes (LTs). It is now recognized that there are two closely related cyclooxygenase enzymes referred to as COX I and COX II. The COX I enzyme is the constitutive form and COX II is the inducible form of the enzyme (45). Intense efforts have failed to show a similar enzyme pattern for the LOX pathway; however, there may be other enzyme systems such as the nitrous oxide pathway that have both a constitutive and an inducible form. These finding have far reaching implications for understanding the biology and physiology of complex biochemical cascades and responses to pathologic stimuli and/or autoimmune diseases, and for the development of interventional drugs with great specificity in the treatment of diseases such as arthritis.

There is a large number of both internal and external stimuli including radiation that trigger the release of AA from membranes. AA release and subsequent metabolism through the AA cascade is related to the action of phospholipases A, C and to a lesser extent, D (63). Membrane release and metabolism of AA appears to be a completely separate event compared to the physiological actions of the eicosanoids. Evidence supporting this conclusion comes from the fact that all of the physiological actions of the eicosanoids can be elicited by exogenously administered PGs and LTs. Eicosanoids produced by cells are autocrines that evoke a variety of physiological effects at very low levels. They have short half-lives of seconds to minutes before they "seek out" their receptors and activate a complex series of events. There is abundant evidence that the physiological effects associated with the eicosanoids are induced through signal transduction pathways (62, 63). Eicosanoids bind to one of a superfamily of specific receptors composed of 7 transmembrane elements (30, 44, 67, 68, 77); each with its own G (guanine nucleotide binding) protein. The specificity of this ligand-receptor recognition is shown by the

biological activity of the stereoisomers of the $PGE_1$ analog, misoprostol (3). A 180 degree rotation of the hydroxyl and the methyl groups on the 16 carbon of the omega chain of misoprostol eliminates the activity of this compound. Once ligand-receptor binding occurs through charge attraction and charge orientation (41), the activated G protein splits into $\alpha$ and $\beta\gamma$ components (13). The subsequent second messenger pathway(s) appear to be linked through adenylyl cyclase and cAMP to protein kinases C and A and possibly, tyrosine kinases, and to diacylglycerol (DAG) and/or phosphatidylinositol triphosphate ($PIP_3$) (62). The phosphorylation of phosphatidylinositol is associated with calmodulin activation and $Ca^{++}$ movement that may, in turn, lead to intestinal smooth muscle contraction and diarrhea which is a side-effect of some PGs in rodents, dogs and human populations. There is also evidence that these messengers interact with early response transcription factors *fos* and *jun* (11), but it is not clear how any of the known or proposed pathways or genomic interactions of the signal transducers may translate into the observed physiological effects of eicosanoids.

**EICOSANOID-INDUCED MODULATION OF RADIATION INJURY:**

Robert used the term "cytoprotection" (56) to describe the *in vivo* protective effects that PGs had on tissues when given before a variety of injurious agents including, ethanol, heat, acids, bases, or nonsteroidal anti-inflammatory agents (NSAIDs) (57). These intriguing studies were the impetus for studies in our laboratory on *in vivo* PG-induced radioprotection (26). Although PG-induced radioprotection, and now, chemoprotection are consistently found *in vivo*, neither is consistently found *in vitro*. Prasad (55) was the first to report radioprotection of CHO cells by $PGE_1$ in 1972. Lehnert (34) followed with a similar, but confusing study showing radioprotection of $PGE_1$ treated V-79 cells given lower radiation doses associated with the

shoulder portion of survival curves but sensitization of these cells given higher radiation doses associated with the exponential portion of the curves. No explanation of these results is available. Walden et al. (73) reported increased survival of irradiated V-79 cells pretreated with leukotriene $B_4$. In contrast, Millar and Jinks (42) failed to find radiation protection of V-79 cells by $PGA_1$ and Holahan et al. (29), also using V-79 cells did not find any protection by $PGE_2$. Likewise, Rubin et al. (59) did not find *in vitro* protection of bovine endothelial cells using a variety of PGs including the analogue, 16-16 dm $PGE_2$. In these studies, PG receptors were greatly reduced or missing from the cultured cells which likely explained the lack of protection since it has been demonstrated that cytoprotection and radioprotection are dependent upon PG receptors (23). Studies reported by Zaffaroni et al. (79) showed that a PG analog, nocloprost, protected normal human fibroblasts *in vitro* but this analog did not protect a human colon tumor cell line. Studies by Sankaranarayanan, et al. (61) showed that PGs did not protect cells grown as monolayers but they did protect the same cells grown as spheroids which is consistent with previous results (21). Most recently, van Buul, et al., (71) showed that misoprostol protected transfected cells which expressed the receptor for $PGE_2$ but misoprostol had no effect on nontransfected cells which did not express the same receptor. This is strong evidence that cellular differences in the expression of receptors likely accounts for the observed differences in PG-induced radioprotection *in vitro*.

Unlike the apparent variability between *in vitro* studies, PG-induced radiation protection *in vivo* has been consistently found (5-7, 17-20, 22, 26, 39, 61, 65, 66, 70, 74-76). Eicosanoids have been shown to protect cells of the intestine, bone marrow, and hair follicles from radiation injury. The $E_2$ analog, 16,16 dm $PGE_2$ protects hair follicles from both single and fractionated

radiation at clinically relevant doses of 2-3 Gy/fraction (12, 25, 40). Although the mechanism of eicosanoid-induced radioprotection is not well understood at the moment, progress is being made. Studies by van Buul and colleagues on DNA repair proficient and deficient cell lines grown as spheroids (7, 61, 70) (71) suggested that PGs did not protect repair deficient cells. Furthermore, while the $PGE_1$ analogue, misoprostol, protected normal spermatogonial cells *in vivo*, misoprostol did not protect spermatogonial cells in mice with severe combined immune deficiency (SCID mice) which have a defect in their DNA repair capability. The same results were found using both cell survival and chromosomal aberrations as end-points. In their most recent studies, van Buul and colleagues (71) showed that P53 status did not influence PG-induced radioprotection and also showed that misoprostol preferentially protected cells in the $G_1$ stage of the cell cycle with little or no protection in the S-phase. Recent unpublished results from our laboratory also suggest that PGs (16-16 dm $PGE_2$ or misoprostol) did not influence radiation-induced apoptosis in either murine intestinal crypts or hair follicles at low doses of $^{137}Cs$ gamma radiation.

**EICOSANOID-INDUCED PROTECTION FROM ONCOGENIC TRANSFORMATION:**

Agents, both within our environment and those used to treat diseases, are known to be carcinogenic to varying degrees. As the ability to treat certain types of cancer increases, the susceptibility of cancer survivors to secondary cancers will continue to increase. The aminothiols are recognized as "chemopreventative" agents that reduce radiation mutagenesis in both *in vitro* (15) and in *in vivo* rodent models (16, 28). Clinical trials to test the ability of Amifostine to prevent secondary tumors are in the planing stages. Although it is clear that the thiols and the eicosanoids protect cells through completely different mechanisms (18), it was of great interest to

investigate the possibility that eicosanoids might also reduce oncogenic transformation. Richard Miller et. al (33, 43) investigated the influence of misoprostol on the number of transformed cells in Syrian hamster embryos (SHE) exposed *in utero* to graded doses of X-rays. Misoprostol (1.25 μg/g body weight), given to the pregnant female 2 hrs before X-ray exposures up to 6.0 Gy, protected the SHE cells from cell lethality yielding a protection factor of 1.5 which is about the same degree of protection as that seen in other *in vivo* rodent cell systems. Misoprostol also protected the SHE cells from X-ray-induced transformation to a remarkable degree yielding a consistent protection factor of about 20 (43). This is the largest protection factor from radiation-induced oncogenic transformation that has been reported. These data suggest that the eicosanoids may be useful in environmental and clinical settings to prevent or reduce the risk of cancer. The available data also beg the question of whether the thiols or the eicosanoids (or perhaps a combination of the two) may be useful in the future in prophylactically reducing oncogenic transformation from well known carcinogens such as tobacco or the sun.

**MODULATION OF RADIATION INJURY BY CYTOKINES:**

Cytokines include a wide variety of hormone-like agents with a concomitant wide variety and bewildering array of cellular actions. Cytokines have a broad spectrum of chemical structures with different cellular origins and with different target cells. Their common link with one another is their effects on regulatory processes of cell growth and cell functions which ultimately is orchestrated to maintain homoeostasis. Either constitutive or induced cytokines may function as autocrines, juxtacrines, paracrines and/or endocrines. Several cytokines have been found to have an influence on radiation injury, but it is important to separate radioprotection from the influence of cytokines on the recovery of tissue after radiation injury.

**CYTOKINE-INDUCED RADIOPROTECTION:**

Some members of the family of cytokines function as radioprotectors; for example, Neta, et al. (47-49, 51) reported on the radioprotective effect of interleukin-1 (IL-1) in mice. Since these studies in the mid 1980s, a variety of cytokines have been found to modulate radiation injury (46). Like most classic radioprotectors, cytokines that protect from radiation must be given before radiation to be very effective. In addition to IL-1; IL-11, IL-12 (50) and IL-15 have all been shown to protect or to be involved in pathways associated with the protection of the hemopoietic cell renewal system. The interleukins must be given between 18 and 24 hours before radiation to provide the maximum protection which suggests the possibility, if not the likelihood, that protection results from the induction of secondary mechanisms or cascades of pathways that lead to protection. For example, IL-1 has been shown to increase cellular levels of the antioxidant enzyme, manganese superoxide dismutase (MnSOD) which may contribute or be responsible for IL-1-induced protection (8, 9, 32). Other cytokines, including tumor necrosis factor (TNF-alpha) (31), mast cell growth factor [(MGF) also know as c-kit ligand, Steel factor or stem cell factor] (53) also protect the hemopoietic tissue. In contrast, TGF-beta, IL-6, and interferons have been shown to sensitize the hemopoietic system to radiation (46). TGF-beta is specifically associated with the pathogenesis of the late sequelae of radiation injury; particularly fibrosis (2).

While most of the work on the protective effects of cytokines has been done on the radiosensitive hemopoietic cell renewal system, IL-1 (58, 78), IL-11 (52, 54) and stem cell factor (SCF) (35), have been shown to protect jejunal crypt cells from radiation as well as increasing animal survival in the range of radiation doses associated with the gastro-intestinal syndrome.

Interestingly, IL-12, while protective of the hemopoietic cell system, sensitizes the intestine to radiation (50). In addition, basic fibroblast growth factor was reported to protect the lungs of mice from radiation-induced pneumonitis, possibly by protecting the endothelial cells from apoptosis (10); however, these findings were not confirmed in other studies (69).

**CYTOKINE-INDUCED RECOVERY FROM RADIATION INJURY:**

Several cytokines including Granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor GM-CSF (36), MGF and IL-3 (53), IL-6 (38), have all been shown to stimulate the recovery of hematopoietic cells following radiation. Patchen points out; however, that *in vitro* results showing increased proliferation of cells treated with MGF the IL-3 were not translated into the same effects in mice and cautioned against the translation of *in vitro* results into clinical studies without more pre-clinical studies (53).

The advantages of the use of G-CSF and/or GM-CSF are quite clear in humans in accelerating the recovery of the hematopoietic cell renewal system after radiation and/or high dose chemotherapy in bone marrow transplantation patients. These results fostered research into synthetic cytokines which might offer increased advantages. Evidence suggested that these "synthokines"; and, in particular, a compound labeled SC-55494 along with G-CSF, was effective against both neutropenia and thrombocytopenia in irradiated non-human primates (37).

**SUMMARY:**

Research on eicosanoids and the cytokines are both difficult and rewarding. It is clear that these compounds are interwoven into the most fundamental physiological processes of life. They are interrelated within their families and with a wide variety of cellular functions.

The eicosanoids exist throughout most of the phylogenetic tree, and as such, their

functions likely evolved early and are fundamental to the normal physiology of cells and tissues. It appears that one of the fundamental roles they play is the rapid response of cells to injury in general. In the cases of repeated insult or chronic injury, the eicosanoids may alter cells to increase survival. While the exact mechanism(s) of how certain PGs exert their protection on cells is unknown, it is clear that protection is mediated through a series or cascade of events. When exogenous PGs are given, it is likely that cells are being "fooled" into a defensive physiological profile. Certain pathological conditions such as arthritis or Crohn's disease produce chronic injury to joints and the bowl respectively and these conditions are associated with the production of large quantities of PGs. They may serve the same function of trying to protect cells during these chronic injury conditions, and they produce pain as well which can be viewed as a protective mechanism for the organism. Some PGs are also chemo-attractants and stimulate an inflammatory response in tissues. Radiation therapy in a clinical setting is a repeated insult and PGs produced in response to radiation injury during the early fractions may play a role in the effects of later fractions or on the recovery of tissues during and after the end of treatment. PGs may also be useful in the prevention of normal tissue damage during radiation therapy. One clinical trial showed that, if properly executed, misoprostol could reduce the degree of mucositis in head and neck cancer patients given standard radiation treatment in 1.8-2 Gy fractions (24). A Radiation Therapy Oncology Group (RTOG) study is underway to further study the possible role of misoprostol in the reduction of radiation-induced mucositis. Additional clinical trials are underway or in the planning stage to investigate the utility of PGs in cancer treatment. PG-induced protection of normal tissues during cancer therapy is only possible provided that tumors are not protected as well. To date, evidence suggests that misoprostol does not protect several

experimental tumors (27); however, tumor protection will be a constant clinical concern.

Sorting out the roles and the interplay of the cytokines in radiation injury is most challenging. It is clear; however, that the clinical utility of some growth factors are well established and the future development of "synthokines" with specific profiles and fewer side-effect will offer clinical advantages. It is also very likely that many new cytokines will be discovered over the next several years: some of which may play additional roles in the modulation of radiation injury.

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