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Review

Eicosanoids and Radiation

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INTRODUCTION

THE MAXIMUM dose of radiation that can be given during radiotherapy depends on the tolerance of the critical normal tissue within the radiation field. Consequently, any attempt to improve the efficacy of radiotherapy must be aimed at increasing tumour radioresponse and/or reducing normal tissue complications. To achieve this objective, many diverse approaches have been investigated, including modulation of levels of eicosanoids, especially prostaglandins (PGs), in both normal tissues and tumours.

Eicosanoids are produced in response to a variety of cellular injuries, and exogenous eicosanoids have been shown to modulate injury induced by a wide array of agents. Therefore, it was logical to infer their involvement in tissue injury by ionising radiation. During the past 20 years, there has been significant research into the role of eicosanoids, primarily PGs, in radiation damage. Attempts have been made to modulate normal tissue damage by administering exogenous PGs or inhibiting endogenous PGs. Other studies have been conducted to increase tumour response to radiation by modulating PG levels in tumours. This overview considers major findings on the role of eicosanoids in the expression and modification of radiation damage, with special emphasis on its therapeutic potential.

EICOSANOIDS

Eicosanoids is a collective term for PGs, thromboxanes (TXs), leucotrienes (LTs), and various hydroxy and hydroperoxy fatty acids. They are metabolites of polyunsaturated fatty acids, such as arachidonic acid, and are produced by virtually all tissues via the cyclo-oxygenase (for PGs and TXs) and lipoxygenase (for LTs) pathways. These end products of the arachidonic acid cascade are bioactive substances; for example, there is an array of PGs: PGE_1, PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin), etc., each exhibiting different biological actions. It is no wonder then that eicosanoids display an astonishingly wide range of pharmacological, physiological, and pathological effects. They play a regulatory role in many physiological homeostatic processes including immunomodulation, vasomotility (constriction and dilatation) and platelet aggregation, along with regulating cell growth and differentiation. They are also implicated in the pathogenesis of a number of pathophysiological syndromes and diseases such as inflammation, rheumatoid states, asthma, autoimmune diseases, and even cancer.

Cells do not store eicosanoids but rather rapidly synthesise them in response to physiological signals including those triggered by various types of cellular injury. Eicosanoids may act on cells that produce them or on neighbouring cells by activating signal transduction processes, such as those involving protein kinase C. They are active in tiny quantities and act rapidly upon release by binding to high-affinity membrane receptors; their action is usually confined to local tissues. Thus, eicosanoids are autocoids (short-lived, local acting substances) and not hormones in the classical sense. Eicosanoids may also act as second messengers and may modify the activity of intracellular enzymes and ion channels.

Although some eicosanoids are produced predominantly by certain specific types of cells, such as PGI_2 by endothelial cells and TXB_2 by platelets, most cells produce many different eicosanoids that may have complementary or antagonistic activities. As a consequence, the effects of eicosanoids on tissues may be quite complex depending on the balance of similar or opposing actions. As an example, while TXA_2 and PGE_2 induce platelet aggregation and are vasoconstrictors, PGI_2 and PGE_1 inhibit platelet aggregation and dilate blood vessels.

EICOSANOID PRODUCTION AFTER IRRADIATION

Ample evidence is available showing that ionising radiation alters eicosanoid production by both mammalian cells *in vitro* [1–3] and tissues irradiated *in vivo* [4–6]. Production increases, decreases, or remains unchanged depending on cell type or tissue, radiation dose, and time after irradiation when the eicosanoids are measured. Most information from *in vitro* studies relates to PGI_2 production by irradiated endothelial cells. PGI_2 release increases within 24 h after irradiation and then falls to normal or below normal several days or weeks after irradiation. In general, higher radiation doses cause more profound changes. Evidence suggests that the early rise in PGI_2 is the result of *de novo* synthesis as the radiation generates oxygen radicals and peroxides, which in turn release PG substrates from the cell membrane stores and activate the PGH_2 synthetase enzyme complex. The relationship between radiation-induced increases in PG release and radiation-induced cell lethality is unclear. The amount of released PGs increases as the number of cells lysed by irradiation is increased, but there is no evidence that released PGs enhance *in vitro* cell killing by radiation. In fact, certain

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eicosanoids were radioprotective when added to cultured cells prior to irradiation [7–9].

Reports on the *in vivo* effects of radiation on eicosanoid production are more numerous than those from *in vitro* studies. Virtually every tissue and organ of experimental animals (mostly rats and mice, although larger animal species have also been used) has been assayed for PGs production after partial or total body irradiation. Radiation was commonly given as a single dose ranging from 2 to 30 Gy; fractionated irradiation was rarely given. PGs were determined in tissue homogenates, organ perfusates, plasma, or urinary excretions, usually within hours or days after irradiation, but some studies covered weeks or even months after exposure.

On the basis of the available data, recently tabulated in detail by Michalowski [10], it is difficult to construct a unified picture of eicosanoid biosynthetic responses to radiation in tissues; however, some general conclusions can be drawn. Within hours after irradiation, most tissues have increased levels of PGs and TXs: PGE_1, PGE_2, $PGF_{2\alpha}$, PGI_2, TXA_2, and TXB_2. In most instances, the elevation is one to several-fold above control values. PG levels appear to be higher after higher doses of radiation, although well-established radiation dose–response curves are not available. Tissue levels of PGs remain elevated for several days, and sometimes for weeks after irradiation. Based on findings from irradiated blood vessels, it appears that the kinetics of PGI_2 responses differ from those of other PGs: a several-fold increase in PGI_2 occurs several days after irradiation, which is followed by a substantial decrease during the following weeks and months. In man, total body irradiation (11 fractions of 1.2 Gy/fraction in 4 days) causes a 3-fold increase in urinary excretion of 6-keto $PGF_{1\alpha}$, the stable metabolite of PGI_2, when measured upon completion of the treatment.

What is the significance of the release of much higher than normal quantities of PGs? Since they are highly potent biological substances, it is difficult to imagine them simply as markers of tissue injury. Rather, it is likely that they actively modify both pathogenesis and expression of radiation-induced tissue damage. However, their regulatory role may be quite complex because the many PGs and LTs simultaneously released in the same tissue have different biological actions, some being agonistic and some antagonistic. For example, released TXB_2 stimulates development of thrombosis in irradiated blood vessels by promoting platelet aggregation, especially at the site of damaged endothelial cells, and by its vasoconstrictive effect. Consequently, the cells in tissues supplied by these blood vessels would lyse; the process amounts to indirect cell kill by radiation. In contrast, released PGI_2 would act in the opposite direction. Therefore, the net result of tissue injury may be dependent, in part, on the interaction of different eicosanoids. In reality, the situation is even more complex because the released eicosanoids can initiate release of other biologically active substances, such as cytokines and growth factors, that could on their own enhance or reduce the expression of radiation damage. However, the indirect evidence that this acute release of large amounts of eicosanoids, in general, promotes radiation injury is substantiated by clinical evidence [11] and by animal research findings [10], which have shown that some normal tissue injuries and complications after irradiation can be ameliorated by treatment with PG synthesis-inhibiting agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticosteroids.

AMELIORATION OF NORMAL TISSUE DAMAGE BY PG-INHIBITING AGENTS

NSAIDs are chemically heterogeneous compounds that have the common property of inhibiting PG and TX production by inhibiting the cyclo-oxygenase pathway. Some also inhibit LT production by blocking the lipoxygenase pathway. Glucocorticoids inhibit production of all eicosanoids because, in addition to their ability to inhibit cyclo-oxygenase, they prevent release of arachidonic acid from membrane phospholipids by stimulating the generation and secretion of lipocortins. The NSAIDs, and especially the glucocorticoids, have been successfully used to reduce both acute and chronic radiation damage of different tissues and organs in experimental animals and man [10, 11]. To illustrate, we will cite only a few examples here. Daily treatments with cortisone attenuate and shorten acute skin reactions including erythema, moist desquamation and ulceration. Radiation-induced pneumonitis can be successfully treated with glucocorticoids and with some NSAIDs such as oxyphenbutazone. Radiation injuries to the alimentary tract, such as to the oral cavity, oesophagus, or colon, can be prevented or reduced by indomethacin and other NSAIDs. Treatment with indomethacin protects haematopoietic tissues from radiation injury by stimulating proliferation of haematopoietic stem cells and by accelerating the rate of their recovery.

NORMAL TISSUE PROTECTION BY EICOSANOIDS

In contrast to normal tissue protection by agents that block eicosanoids, an apparent paradox is presented by data showing that certain eicosanoids can be radioprotective both for cultured cells and for tissues *in vivo*. This was first observed for PGE_1, which increased survival of CHO [7] and V-79 cells [8] when added to the culture medium prior to cell irradiation. Although the mechanism of protection remains unknown, the protective effect was attributed to PG-induced elevation of cyclic AMP. These findings have been contested since the addition of neither PGE_1 nor PGA_2, nor cell treatment with flurbiprofen, an inhibitor of PG synthesis, modified the radioresponse of V-79 cells or some other cell lines [12]. Also, 16, 16-dimethyl PGE_2, a synthetic analogue of PGE_2, failed to radioprotect V-79 cells that had been first depleted of glutathione [13]. The interpretation was that glutathione either masked prostaglandin receptors or that, on its own, it provided maximum protection. In addition, 16, 16-dimethyl PGE_2 failed to protect M-1 melanoma cells when grown as a monolayer, but it was radioprotective when the cells were grown as spheroids [14]. More recently, misoprostol, a synthetic analogue of PGE_1, was found to be highly potent (by 20-fold) in preventing radiation-induced oncogenic transformation of Syrian hamster embryo cells when treated *in utero* and assayed *in vitro* [15]. Survival of these cells, however, was increased by only a factor of 1.5. An interesting observation was recently reported that nocloprost, a PGE_2 analogue, protected human fibroblasts but not a colon adenocarcinoma cell line against killing by radiation [16]. This implies that some eicosanoids may be protective for normal tissues but not tumours, which might be used advantageously in radiotherapy. The leukotriene, LTC_4, was also found to render radioprotection to V-79 [9] but not to bovine aortic endothelial cells [17]. Overall, this relatively limited and largely inconsistent information shows that some eicosanoids may act as protectors against radiation damage *in vitro*, but the exertion of such an effect depends on many, still poorly defined conditions. Nevertheless, consistent observation was that eicosanoids rendered radioprotection only when given before irradiation.

In vivo studies have shown more consistent radioprotective activity of eicosanoids than those *in vitro*. Interest in *in vivo* radioprotection with these agents stemmed from the observations made by Robert and associates [18, 19] that PGE_2, 16, 16-dimethyl PGE_2, and some others protected gastric or

intestinal mucosa from injury inflicted by acids, bases, alcohol, heat and NSAIDs. In the first radioprotection study, 16, 16-dimethyl PGE_2 given subcutaneously (s.c.) protected intestinal crypts from damage by radiation given 1 h later [20]. Since then, a variety of eicosanoids, including PGE_1, PGE_2, PGA_1, PGA_2, $PGF_{2\alpha}$, PGI_2, iloprost, misoprostol and LTC_4, have been identified as having varying degrees of radioprotection for intestine [21], bone marrow [22–24] and hair follicles [25–27]. The protection occurred only when the agents were given within hours before irradiation, which in almost all studies was delivered as a single dose. Systemic or topical applications of 16, 16-dimethyl PGE_2 were effective in protecting mice from alopecia after fractionated irradiation [26]. The agent applied 1 h before each daily fraction given for 5, 10, or 15 days increased hair regrowth by 25 to 100%. Figure 1 shows three important details of PG-induced radioprotection of intestinal clonogenic cells. Firstly, PGs protected in microgram quantities and secondly, there was a rapid increase in protection at low PG doses, with the maximum protection being achieved at approximately 10 μg/30 g mouse (0.3 mg/kg). Thirdly, there was a wide range of protection, PGE_1 providing no protection and misoprostol and iloprost providing the maximum protection. It is interesting to note that the PG analogues were more protective than the naturally occurring structures. For nearly all PGs investigated to date, the maximum radioprotection was seen when the PGs were given 1–2 h before radiation.

Just how eicosanoids protect cells and tissues against injury by an agent, including radiation, is not known. It appears that many types of cells have highly specific receptors for these substances to which they bind [28–31]. If cells do not have receptors or if they are blocked, protection cannot be achieved [17]. Receptor binding activates transduction pathways involving G-proteins, cAMP and protein kinases [32], and there is evidence that the transduction signals interact with *FOS* and *JUN* in the cellular genome under some conditions [33]. How some or all of these molecular messages are translated into radioprotection is not known. There are some indications that protection might involve stimulation of DNA repair [34]. In addition, it appears that glucocorticoids are involved in the mechanism of radioprotection induced by certain eicosanoids, as adrenalectomy abolished protection of jejunal crypts by the E-series PGs but not by iloprost or LTC_4 [35]. Radioprotection was restored by cortisone administration. It is not known how glucocorticoids perform this activity.

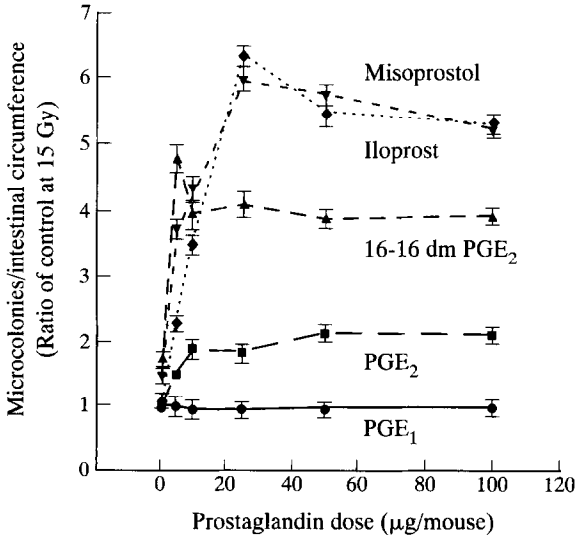

Figure 1. Microcolonies per jejunal circumference as a relative ratio of controls at 15.0 Gy versus dose of several prostaglandins. The data are the mean values from five mice ±1 S.E.M.

Another approach to radioprotection consisted of administering unsaturated essential fatty acids to provide a sufficient amount of precursors specifically or preferentially for eicosanoids having desirable effects, such as PGE_1. PGE_1 inhibits platelet aggregation, dilates blood vessels, inhibits inflammatory reactions [36] and, as shown above, is a cellular radioprotector in some systems. An increased level of PGE_1 can be achieved by giving gamma-linolenic acid (GLA) and eicosapentaenoic acid (EPA) [36–39]. The stimulation of the pathway leading to PGE_1 can, in turn, block production of PGE_2 and TXA_2, either of which may have deleterious effects on normal cells. Furthermore, these acids lead to another metabolite, 15-OH-dihomo-gamma-linolenic acid, which blocks production of LTs [39]. Therefore, administration of GLA and EPA may not only enhance production of PGs that may be beneficial, but also simultaneously inhibit deleterious platelet aggregative and pro-inflammatory eicosanoids. A recent study has clearly demonstrated that daily oral administration of GLA and EPA in the form of oils reduced both early and late damage to pig skin induced by single or fractionated irradiation [38]. The early damage, consisting of bright red erythema and moist desquamation, was reduced by factors of 1.06–1.24, and late damage, consisting of dusky/mauve erythema and dermal necrosis, was reduced by factors of 1.14–1.35. The protection was achieved only when the acids were given over the time course of radiation and development of radiation damage. No protection was seen when the acids were given prior to irradiation, which is opposite to the protection achieved with eicosanoids (see above).

EICOSANOIDS AND TUMOURS

Eicosanoids, in particular PGs, have been implicated in the regulation and the initiation and promotion steps in tumour development, and in the rate of tumour growth and dissemination [40–43]. Tumours in man and experimental animals, in general, produce excessive amounts of PGs, although the production varies both quantitatively and qualitatively among tumours of different histological types, and among individual tumours of the same histology. An example of this wide variability is shown in Table 1 for murine tumours. Of five murine tumours, three produced PGs which, in addition, varied widely in the type and amount produced. Similarly, the tumours varied widely in their production of LTs. Most production of eicosanoids in tumours is from tumour cells; however, infiltrating normal cells, such as macrophages, lymphocytes, fibroblasts, and endothelial cells, can participate in eicosanoid production. Because different types and quantities of eicosanoids are produced, the biological action will primarily depend on the type and quantity of eicosanoids produced. Although some eicosanoids possess inhibitory [40, 43] and some stimulatory [40, 41] properties for tumour cell growth, most information available thus far implies that tumour-produced eicosanoids are associated with enhanced tumour growth and metastatic spread [42].

Inhibition of PGs by indomethacin or similar agents frequently results in antitumoral and antimetastatic activity [44–46]. The activity is commonly expressed as a retardation in tumour growth rate, whereas complete and lasting tumour regressions are extremely rare. The mechanisms underlying this effect are most often considered to be immunological, whereby indomethacin would eliminate immunosuppressive PGs, notably

Table 1. Eicosanoid metabolites of mouse tumours

Eicosanoid	Mouse tumours				
	FSA	NFSA	SA-NH	MCA-K	HCA-I
$6KPGF_{1a}$	102.8*	135.1	3.7	ND†	10.3
TXB_2	46.2	31.9	ND	ND	28.6
PGF_{2a}	ND	11.3	ND	ND	24.3
PGE_2	336.1	36.6	ND	ND	14.5
PGD_2	ND	ND	ND	ND	6.2
LTB_4	ND	28.9	10.7	64.0	ND
HHT	83.5	35.4	2.8	14.7	45.7
di-HETEs	ND	ND	12.1	65.6	ND
15-HETE	ND	19.3	ND	ND	ND
5- or 12-HETE	146.7	30.5	26.6	86.9	115.7
Total metabolites	715.3	329.0	55.9	231.2	245.0

Tumours were generated by 5×10^5 tumour cells injected into the muscles of the right thighs and were assayed for eicosanoid metabolites when approximately 9 mm in diameter.

*Values in ng/g are given as the mean of two samples. Each sample was obtained from a pool of 4–6 tumours; †ND, not detectable. Reprinted from [46] with permission.

PGE_2 [47, 48]. Our own studies revealed that a major mechanism of indomethacin-induced tumour retardation is inhibition of PG-mediated tumour neovascularisation [47, 49]. We further observed that the antitumour effect of indomethacin depended entirely on the tumour's ability to produce PGs: only tumours that produced PGs responded to indomethacin. The finding might have a valuable clinical application in that it may be possible to predict which tumours will respond to therapy with PG-inhibitory drugs, by assaying the PG profile of individual patients prior to therapy.

INHIBITION OF PROSTAGLANDINS AND TUMOUR RADIORESPONSE

Besides having a direct antitumour effect, there is evidence that PG-inhibiting agents can augment tumour response to radiotherapy, both in experimental cell and animal systems [47, 50–52] and in the clinic [53]. Early studies attributed this effect to the agents' reduction of the inflammatory response to radiation [50]. In our own studies, we initially hypothesised that PGs in tumours act as radioprotectors, and, therefore, their removal would increase cellular radiosensitivity [52]. Indeed, treatment of mice with indomethacin greatly lowered PG levels in tumours and significantly increased tumour radioresponse, manifested in the prolongation of tumour growth delay, increase in tumour cure rate, and in delay of post-irradiation recurrences [47, 52]. The potentiation of radioresponse ranged from a factor of 1.3 to more than 2, which depended on many factors including tumour type, choice of fractionation in radiation treatment schedule, timing of indomethacin administration in relation to tumour irradiation and the endpoint of tumour response. It was greater with fractionated than with single-dose irradiation [47]. In most instances, indomethacin was given in the drinking water for 10 days starting 2–3 days before tumour irradiation. Figure 2 illustrates the indomethacin-induced potentiation of radioresponse of two murine sarcomas.

Our studies further revealed that a major mechanism of increased radioresponse was not through the removal of radioprotection, but rather the removal of the immunosuppressive

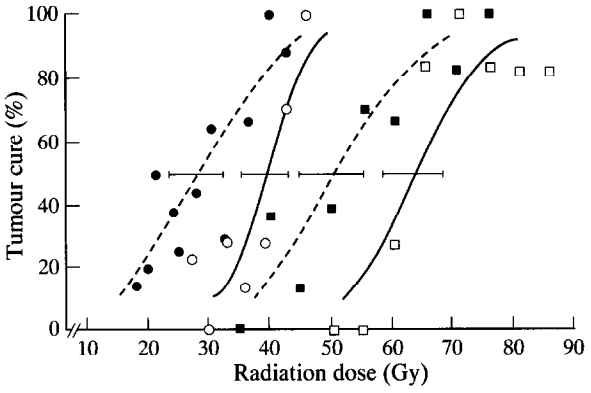

Figure 2. Radiation dose–response curves for local tumour control of FSA (circles) or NSFA tumours (squares). Open symbols, irradiation-only groups; solid symbols, indomethacin plus irradiation groups. Error bars at the TCD_{50}, 95% confidence limits. Treatment with indomethacin, 35 μg/ml in drinking water, was started when tumours grew to 6 mm in diameter and was continued daily for 10 days. Local tumour irradiation was given to 8 mm tumours. Reprinted from [52] with permission.

action of PGs: indomethacin was effective even when given after tumour irradiation was complete, while its effect was reduced in immunocompromised mice [47]. Overall, this treatment significantly improved the therapeutic ratio because, under similar conditions, it did not influence the radioresponse of a number of normal tissues [52, 54] or protected some, such as the haematopoietic tissue [54, 55] and the lung [54].

CONCLUSION

The eicosanoids are a large, diverse and potent family of bioactive autocrines with a large array of actions on cell and tissue functions, many of which are associated with the preservation of homeostasis. Eicosanoid synthesis and subsequent tissue alterations in physiological regulation can be triggered by a variety of factors and conditions associated with injury in general; it is no wonder then that there is an association between eicosanoids and radiation injury. The association, however, remains largely undefined because of the complexity of the interactions brought about by the simultaneous release of numerous types of eicosanoids with different and often opposing actions. In spite of this complexity, certain generalisations can be made.

In response to radiation, large amounts of eicosanoids are released from cells and tissue that, in turn, can alter the profile of the cellular response to injury. Eicosanoid synthesis may be associated with the release of other classes of biologically active agents such as cytokines, growth factors (either growth stimulatory or inhibitory) or cytotoxic substances. Alternatively, eicosanoids may initiate production of some of these other substances. This complex array of bioactive agents is associated with, or causes, acute tissue responses manifested by inflammatory reactions such as radiation-induced enteritis and pneumonitis. These conditions can be successfully controlled by eicosanoid-inhibiting agents, including both corticoids and NSAIDs. Procoagulant and proinflammatory eicosanoids (e.g. some TXs and LTs) and/or radiation-induced decreases in PGI_2 may participate in and elevate radiation injury of blood vessels, leading to late injury. Shifting the metabolism of arachidonic acid towards production of protective PGs, such as PGE_1, by administering GLA or EPA can ameliorate late injury in some tissues. Very importantly, evidence suggests that eicosanoid-

induced protection from late injury can be achieved by initiating the treatment once the irradiation is completed. This targeted "radioprotection" warrants further investigation.

Several natural PGs and a number of their synthetic analogues have the ability to protect cells and tissues from radiation injury, but to do that they must be given before radiation. The mechanism of action associated with this protection is unknown, but it is clear that it is quite different from the mechanism of protection by the aminothiols [56], another potent class of radioprotective agents. In clinical settings, the eicosanoids alone or in combination with some forms of aminothiols or other protectors with different mechanisms of action may provide a high degree of normal tissue protection during cancer therapy. The success of this strategy for increasing the therapeutic gain depends upon the important assumption that these protectors have no influence on the radiation response of tumours. Unfortunately, little is currently known regarding any effect of PGs alone or combined with other agents on the radiation response of tumours.

Since many tumours produce large amounts of different eicosanoids, a logical concern is that PGs may act as natural radioprotectors, increasing tumour radioresistance. A limited number of available studies showed that NSAIDs can increase tumour radioresponse, and according to our own findings, this is associated with the inhibition of PGs in tumours. The mechanism of increased tumour radioresponse appears to be due to removal of immunosuppressive actions of PGs; the effect can be achieved by applying NSAIDs after tumour irradiation. As this field of research unfolds, it appears possible that the most effective way to increase the therapeutic gain may be to give combinations of protectors (that do not protect tumours) along with NSAIDs (alone or combined with other tumour sensitisers) to both protect normal tissue and sensitise tumours to cancer treatment. Expansion of research on the protective role of eicosanoids in tumour radioresponse is needed, and application of NSAIDs in tumour radiotherapy is justified.

Modern radiobiology recognises that different autocrine and paracrine cell growth regulatory substances, cell receptors, and transduction pathways, as well as gene products, play important roles in the regulation of the expression and repair of cell injury by radiation [57], and there is no doubt that eicosanoids represent an important aspect of this regulation. Recent rapid developments in molecular technology now allow investigations into the mechanisms of eicosanoid actions. As each is delineated, it is conceivable that, through gene regulation or manipulation, it may be possible to influence the normal and tumour cell production of those eicosanoids that prove to lessen normal tissue injury or increase tumour cell cytotoxicity by radiation or other treatment modalities.

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