Radioprotection: the non-steroidal anti-inflammatory drugs (NSAIDs) and prostaglandins

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Abstract

Clinical and experimental studies of the acute and late effects of radiation on cells have enhanced our knowledge of radiotherapy and have led to the optimisation of radiation treatment schedules and to more precise modes of radiation delivery. However, as both normal and cancerous tissues have similar response to radiation exposure, radiation-induced injury on normal tissues may present either during, or after the completion of, the radiotherapy treatment. Studies on both NSAIDs and prostaglandins have indeed shown some evidence of radioprotection. Both have the potential to increase the survival of cells but by entirely different mechanisms. Studies of cell kinetics reveal that cells in the mitotic (M) and late G2 phases of the cell cycle are generally most sensitive to radiation compared with cells in the early S and G1/G0 phases. Furthermore, radiation leads to a mitotic delay in the cell cycle. Thus, chemical agents that either limit the proportion of cells in the M and G2 phases of the cell cycle or enhance rapid cell growth could in principle be exploited for their potential use as radioprotectors to normal tissue during irradiation. NSAIDs have been shown to exert anti-cancer effects by causing cell-cycle arrest, shifting cells towards a quiescence state (G0/G1). The same mechanism of action was observed in radioprotection of normal tissues. An increase in arachidonic acid concentrations after exposure to NSAIDs also leads to the production of an apoptosis-inducer ceramide. NSAIDs also elevate the level of superoxide dismutase in cells. Activation of heat shock proteins by NSAIDs increases cell survival by alteration of cytokine expression. A role for NSAIDs with respect to inhibition of cellular proliferation possibly by an anti-angiogenesis mechanism has also been suggested. Several in-vivo studies have provided evidence suggesting that NSAIDs may protect normal tissues from radiation injury. Prostaglandins do not regulate the cell cycle, but they do have a variety of effects on cell growth and differentiation. PGE_2 mediates angiogenesis, increasing the supply of oxygen and nutrients, essential for cellular survival and growth. Accordingly, PGE_2 at sufficiently high plasma concentrations enhances cellular survival by inhibiting pro-inflammatory cytokines such as TNF-α and IL-1β. Thus, PGE_2 acts as a modulator, rather than a mediator, of inflammation. Prospective studies have suggested the potential use of misoprostol, a PGE_1 analogue, before irradiation, in prevention of radiation-induced side effects. The current understanding of the pharmacology of NSAIDs and prostaglandins shows great potential to minimise the adverse effects of radiotherapy on normal tissue.

Introduction

The improved methods for prevention and early detection of malignancy, as well as recent advances in the treatment of cancer, have served to decrease the mortality observed among cancer patients (Bray et al 2002). The most obvious advances are the result of improved surgical techniques combined with cytotoxic radiation and drug therapy.

Although radiation therapy provides therapeutic benefit in cancer patients, physicians are concerned with its side effects. After all, there is also the possibility that patients may develop neoplastic diseases as a consequence of radio- or chemotherapy (Wu et al 1999; Little 2001).

Whether the goal of therapy is palliation or cure determines the degree of risk and cost that the patient and health-care system are willing to accept, since virtually all cytotoxic therapies have a narrow therapeutic index. This toxicity to normal organs not

only limits the use and achievement of full therapeutic potential of cytotoxic agents and radiotherapy but also claims a cost in terms of patient morbidity and mortality (Grant & Dent 2001; Coleman 2002).

In head and neck radiation, the common oral complications include mucositis, infection, salivary gland dysfunction, taste dysfunction and pain. These complications, if unresolved, can lead to secondary complications such as dehydration, dysphagia and malnutrition. Furthermore, radiotherapy can irreversibly injure oral mucosa, vasculature, muscle and bone (Mazeron et al 2002). A widely accepted hypothesis for the observed side effects of radiation is the generation and amplification of free radicals (Mitchell et al 2000; Das 2002).

In radiation therapy, one may mitigate the toxic effects of ionising radiation on normal tissue by adjusting the dose of radiation and frequency of treatment (Coleman 2002). A major drawback of this strategy is insufficient control of the tumour. Hence, there is a growing area for new drug development in the field of supportive care agents, which are intended to avert or minimise treatment-limiting toxicity to normal organs.

One of the approaches to minimise damaging effects of radiotherapy is the use of cytoprotective agents or cytoprotectors (also known as radioprotectors). These drugs, such as amifostine (WR-2721), selectively protect normal tissues from the cytotoxic effects of radiation while preserving their antitumour effects (Koukourakis 2002). Another approach adopted by physicians is the use of palliative agents such as sucralfate suspension, sodium bicarbonate and topical anaesthetics after radiotherapy to relieve certain side effects or symptoms experienced by patients, although the effectiveness of these therapies has recently been questioned (Worthington et al 2002).

Radiolysis has often been considered to be the major mechanism generating reactive oxygen species that lead to tissue damage (Das 2002). Antioxidants confer radioprotection by scavenging free radicals (Weiss & Landauer 2000; Das 2002). The radioprotective properties of antioxidant natural products, such as vitamin E, vitamin A and β carotene and therapeutic agents, such as β-blockers (propranolol), theophylline, calcium-channel blockers (nimodipine) and amifostine, has been demonstrated (Weiss & Landauer 2000). Meanwhile, a stable free-radical nitroxide, Tempol, has been shown to have potent antioxidant and radioprotective properties (Mitchell et al 2000).

It has been suggested that local tissue damage triggers an inflammatory response. Its subsequent repair may generate new tissue or fibrotic replacement. The arachidonic acid cascade is known to generate inflammatory mediators such as histamine, kinins and prostanoids (Wenzel 1997; Kaplan et al 2002). Inhibition of the arachidonic acid pathway will minimise, if not control, the spread of the radiation-induced inflammation.

When cells are damaged beyond repair, apoptosis, a complex and tightly regulated process, is triggered. Apoptosis is defined as a programmed cell death (Granville et al 1998) and is characterised by nuclear DNA fragmentation, chromatin condensation and a characteristic cytoplasmic and nuclear morphology, before the cell is eliminated by phagocytosis. Apoptosis occurs under physiological conditions and also after radio- (and chemo-) therapy.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in the palliative management of cancer (Cherny 2001) for their anti-inflammatory effects. However, recent evidence suggests that NSAIDs are anti-cancer agents (Bode & Dong 2000; Dermond & Ruegg 2001; Husain et al 2002).

Prostaglandins have no anti-inflammatory effects, but they mediate physiological functions such as cytoprotection in the stomach, vascular homoeostasis and water and sodium reabsorption (Smith 1989). Prostaglandin E2 (PGE$_2$) has been suggested to function as a modulator, rather than simply a mediator, of inflammation (Hinz et al 2000). Currently, there remains a controversy pertaining to the use of prostaglandin analogues such as misoprostol as radioprotectants (Hanson et al 1997).

Radiation and tissue damage

Clinical and experimental studies of the acute and late effects of radiation on cells have enhanced our knowledge of radiotherapy and have led to the optimisation of radiation treatment schedules and to more precise modes of radiation delivery (Ebert 1997; Weiss & Landauer 2000; Coleman 2002). However, even with these improvements, radiotherapy has achieved limited success in eradicating cancer. One major reason for this stems from the fact that normal and cancerous tissues have similar responses to radiation exposure (Mitchell et al 2000). Consequently, radiation-induced injury may present during radiotherapy treatment or some time later after the completion of radiotherapy.

An appreciation of the processes of cell response and adaptation to radiation is necessary to understand the problem of radiation-induced toxicity. Radiation results in direct damage to, or mutation of, the cellular DNA, although it has also been shown that irradiation of the cell cytoplasm can induce mutations in cell nuclei by bystander effects (Wu et al 1999). A tumour suppressor gene, p53, which actively participates in the regulation of the cell cycle, can either trigger cellular repair or activate apoptosis, depending on the degree of radiation-induced injury (Gotz & Montenarh 1996; Komarova & Gudkov 2001).

Acute radiation effects are caused by transient suppression of cell proliferation in tissues with a high rate of cell turn-over, such as the bone marrow, epidermis and the mucosal lining of the respiratory and digestive tracts (Bloomer & Hellman 1975).

The time at which these effects (such as myelosuppression, epidermitis and mucositis) are first observed is determined by the time required for maturation of basal precursor cells into functional non-proliferating end cells (Morris 1996).

Late radiation effects are observed in slow turn-over cells. Firstly, it has been shown that the slow proliferation (or turn-over) of parenchymal cells reflects delayed onset of injury (Mathes & Alexander 1996). Secondly, it has been

hypothesised that late radiation effects occur as a result of functional or structural damage to small blood vessels (capillaries, venules and arterioles) leading to disruption of blood supply to the tissues (Mathes & Alexander 1996). There is also a third category of intermediate effects to describe certain types of normal tissue damage that are first manifested about 2–4 months after the end of treatment (e.g., radiation-induced pneumonitis, which may be mild and resolve spontaneously or may become severe and progress to pulmonary fibrosis (Chernecky & Sarna 2000)).

Recent studies have reiterated the role of inflammatory reaction and endogenous cytokine production in the pathogenesis of some types of radiation-induced damage to normal tissue (Simon et al 1995; Chang et al 1997; Bishop-Bailey et al 1998; Diaz et al 1998; Housby et al 1999; Fedorocko et al 2002). Cytokines are soluble mediators that aid in the communication between cells – primarily cells of immunological, haematological and neurological systems. These cytokines include interleukins (ILs), interferons (INFs), colony stimulating factors (CSFs) such as haematopoietic growth factor and others (Borish & Rosenwasser 1996). During inflammation, mononuclear phagocytes release cytokines which promote cellular infiltration and damage to tissue. These cytokines include IL-1α, IL-1β, TNF (tumour necrosis factor)-α, IL-6 and IL-8, which mediate cellular, humoral and allergic inflammation (as reviewed by Borish & Rosenwasser (1996)). In addition to these pro-inflammatory cytokines, anti-inflammatory cytokines like TGF (transforming growth factor)-β and IL-10 are also produced. TGF-β regulates cell growth, stimulates fibrosis and decreases immune response (Borish & Rosenwasser 1996).

The elucidation of cell cycle machinery has contributed to our understanding of cancer progression (Ford & Pardee 1999) and the optimal use of radiotherapy technique to eliminate tumour cells with minimum damage to normal cells (Ebert 1997).

The transition from G1 to S phases of the cell cycle is a critical step in cell proliferation as it determines DNA synthesis and subsequent cell division (Slingerland & Pagano 2000). The cell cycle is governed by a family of cyclin-dependent kinases (cdk) whose activity is regulated by binding of the cyclins, by phosphorylation and by negative regulators (cdk inhibitors: p15, p16, p18, p19, p21, p27 and p57). The cyclins play a central role in the control of cell proliferation through activation of cdk (Resnitzky et al 1994; Morgan 1995). The cdk–cyclin complexes are inhibited by binding with cdk inhibitors, resulting in cell cycle arrest (Peter & Herskowitz 1994). It is this integration of the cdk–cyclin–cdk inhibitor systems that manages the cell cycle at checkpoints G1, S and G2 and accordingly coordinates the cell cycle transitions (Ford & Pardee 1999). It may be appropriate at this juncture to also highlight that cell cycle arrest, through the management at checkpoints, is crucial to allow damage repair, or to limit the injury, to damaged cells (Bernhard et al 1999). Passage through G1 into S phase is regulated by the activity of cyclin D-, cyclin E- and cyclin A-associated cdk. B-type cyclin-associated kinases regulate the G2/M transition.

It has been well established that radiation leads to mitotic delay in the cell cycle (Morris 1996) and cells are temporarily arrested in the G1, S or G2 phases of the cell cycle. The corollary is the accumulation of cells at the G1/S and G2/M boundaries. It has also been found that cells in the mitotic (M) and late G2 phases of the cell cycle are most sensitive to radiation compared with cells in the early S and G1 phases (Morris 1996). The duration of the mitotic delay is directly proportional to the radiation dose and the cell doubling time (defined as the time taken for the cell number in a population to double) (Morris 1996).

The understanding of cell kinetics has led to the use of more effective radiotherapy techniques to achieve better therapeutic outcome (Morris 1996; Bernhard et al 1999). Most malignant tumours are believed to contain a variable population of stationary cells or cells with very slow turnover, in addition to the rapidly proliferating growth fraction (Bernhard et al 1999). It is also known that most cells of normal tissues remain in the quiescent or G0 state (Bernhard et al 1999).

Radiation-induced DNA damage

It has often been assumed that radiation-induced genetic alterations, such as mutation and carcinogenesis, require direct damage to DNA (Grosovsky 1999). However, recent studies have shown that irradiation of the cell cytoplasm can induce mutations in the cell nucleus as well (Wu et al 1999). This observation of mutagenesis after cytoplasmic irradiation provides evidence for a bystander effect, whereby reactive radiation products or damage signals from a cell can migrate to non-irradiated cells thereby producing biological effects (Grosovsky et al 1996; Wu et al 1999; Zhou et al 2000). Several studies suggest that, besides DNA, cell cycle regulatory proteins are also potential targets for radiation-induced cytotoxicity (Grosovsky et al 1996; Wu et al 1999; Mitchell et al 2000; Zhou et al 2000).

Apoptosis, p53 and radiation damage

Apoptosis, a tightly regulated programmed cell death, occurs under normal physiological conditions but is also an important mechanism by which radiation and chemotherapy kill cells (Granville et al 1998; Watters 1999). Several factors, such as the mutated tumour-suppressor gene, activated oncogenes and growth factors, mediate apoptosis (Holbrook et al 1996; Granville et al 1998).

On irradiation, p53 accumulates and binds to specific DNA sequences within the regulatory regions of certain genes. This results in either activation of transcription or silencing of downstream proteins (as reviewed Steegenga et al 1996; Granville et al 1998). The activation of transcription mediated by p53 causes cells to move into apoptosis and promotes cell cycle arrest and DNA repair.

Cellular adaptive mechanisms in response to radiation

The most basic adaptive measure by which cells can minimise or arrest radiation-induced damage is, simply, normal

homoeostasis. Such homoeostatic mechanisms are absolutely essential to maintenance of daily normal cellular functions when cells are in a constant state of oxidative stress (Gonzalez-Flecha & Demple 2000). The normal homoeostatic mechanisms that cells utilise to recover from the acute effects of radiation (Morris 1996) are: firstly, repair of sublethal radiation damage; secondly, recruitment of stem cells from the resting G0 state into actively cycling state; thirdly, a decrease in the cell cycle time for proliferating cells; and finally, repopulation of the irradiated site via migration of cells from surrounding normal tissue.

Recent studies have also revealed that protein kinase C mediates radioprotection in cells by inhibition of radiation-induced apoptosis (Fuks et al 1994; Haimovitz-Friedman et al 1994; Gamble et al 2000). It was found that basic fibroblast growth factor (bFGF) protected endothelial cells against the lethal effects of ionising radiation by inhibiting apoptosis (Fuks et al 1994). A separate study (Haimovitz-Friedman et al 1994) supported this finding, demonstrating that the radiation protection associated with bFGF stimulation was not mediated via an effect on the repair of DNA breaks. Instead, it was a result of activating protein kinase C. Protein kinase C is known to phosphorylate other protein moieties, triggering a cascade of downstream events (Hallahan et al 1991). In fact, bFGF therapy (administered intravenously into an animal model) rescued whole-lung irradiated mice from lethal radiation pneumonitis (Haimovitz-Friedman et al 1994). It can thus be explained that bFGF activation resulted in activating membrane protein kinase C, which then mediated the inhibition of radiation-induced apoptosis in endothelial cells and conferred protection against the lethal ionising radiation (Haimovitz-Friedman et al 1994).

Non-steroidal anti-inflammatory drugs

NSAIDs are widely used for their anti-inflammatory, anti-pyretic and platelet-inhibitory actions. They are commonly used in the palliative management of cancer (Cherny 2001). Recently, studies on NSAIDs have revealed their anti-proliferative effects on colorectal tumorigenesis (Bode & Dong 2000; Dermond & Ruegg 2001; Husain et al 2002).

Their potential use as anti-cancer agents has been widely examined (Alberts et al 1995; Stoner et al 1999; reviewed in Husain et al 2002). Coincidentally, NSAIDs also exhibit radioprotection on normal tissues by arresting the cell cycle at G1 phase (Bayer et al 1979; Northway et al 1980; Goldberg 1986; Pillsbury et al 1986; Furuta et al 1988; Perugini et al 2000).

Anti-inflammatory effects – non-selective cyclooxygenase inhibition

NSAIDs are generally known to inhibit prostaglandin H synthase which has both cyclooxygenase and hydroperoxidase activity (Smith 1989). The cyclooxygenase enzymes catalyse the formation of prostaglandin G2 (PGG_2) from arachidonic acid, and hydroperoxidase catalyses the reduction of PGG_2 to prostaglandin H2 (PGH_2) (Smith 1989). Prostaglandin H synthase exists in two isoforms, cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2). COX-1 is involved in mediating the physiological functions of prostaglandins, whereas COX-2 is primarily associated with pathological states such as inflammation (Marnett et al 1999). An increase in production of prostaglandins and leukotrienes is commonly associated with inflammation (Wenzel 1997; reviewed in Kontogiorgis & Hadjipavlou-Litina 2002). Consequently, the inhibition of COX enzymes by NSAIDs will reduce the formation of pro-inflammatory oxygenated metabolites (Kontogiorgis & Hadjipavlou-Litina 2002). The elevation of arachidonic acids also activates sphingomyelinase, leading to production of an apoptosis-inducer ceramide (Chan et al 1998).

Anti-proliferative effects and apoptosis: cell cycle arrest

The NSAIDs aspirin, indometacin, naproxen and piroxicam have profound anti-proliferative effects on colon cancer cells in-vitro (Hanif et al 1996; Husain et al 2002). They do so by mediating cell cycle arrest and apoptosis. NSAIDs cause a shift in the cell cycle towards a quiescence phase (i.e. an increased proportion of cells in the G0/G1 phases and a decreased percentage of cells in the S phase of the cell cycle), evident from the reduction of cell cycle regulatory cyclins that are necessary for cell cycle progression (Morgan 1995; Hanif et al 1996).

NSAIDs also induce cell death by an apoptotic process that is independent of p53 (Hanif et al 1996; Rahman et al 2000). In fact, it has been proposed that the cell death may be the result of the increased level of arachidonic acid by inhibiting COX enzymes (Chan et al 1998). It was found that treatment with 200 μM sulindac sulfide (an active sulindac metabolite) for 16 h resulted in a 5.1- and 3.4-fold increase in arachidonic acid in two different cell cultures, respectively. It was also shown that indometacin, an NSAID structurally distinct from sulindac, demonstrated similar results to sulindac sulfide in bringing about cell death. Thus it was proposed that NSAIDs affected tumour growth by inhibiting COX activity, causing a build-up of the COX substrate arachidonic acid. The increased level of arachidonic acid subsequently activated sphingomyelinase that leads to the production of an apoptosis-inducer ceramide (Chan et al 1998; Ohanian & Ohanian 2001).

Antioxidant effect and superoxide dismutase

Superoxide dismutase (SOD) is a eukaryotic antioxidant enzyme that is expressed in response to changes in the level of reactive oxygen species (Gonzalez-Flecha & Demple 2000). Its exact mechanism of action is still unknown, but it is capable of converting superoxide anion O_2^- to H_2O_2 (hydrogen peroxide) (Birnboim 1982). Antioxidant properties of SOD, which reduce oxidative and genotoxic stress in cells after UV (ultra-violet rays) exposure, have

been demonstrated (Kimura et al 2000). Accordingly, NSAIDs can elevate the level of SOD in cells (Nivsarkar 2000). The exact mechanism of NSAIDs as antioxidants is thus still unknown, but they do have the potential to minimise cellular injury.

It has been reported that patients with chronic rheumatoid arthritis have a low level of circulating SOD. This level significantly increases with NSAID therapy (Nivsarkar 2000).

Downstream effects – gene expression

NSAIDs inhibit COX (discussed above) and induce apoptosis. However, NSAIDs also have diverse effects on gene expression in response to a pro-inflammatory stimulus. It has been shown that NSAIDs can inhibit cytokine and adhesion factor expression during monocyte activation (Housby et al 1999). NSAIDs also promote expression of heat shock genes (Soncin & Calderwood 1996).

Activation of heat shock proteins by NSAIDs increases cell survival by inhibiting pro-inflammatory cytokine expression (IL-1 and IL-6), involved in initiation of acute phase response, febrile responses and progression of inflammatory disease (Simon et al 1995). Furthermore, it has also been shown that heat shock proteins can prevent heat-induced protein penetration (Michels et al 1997).

In addition to protein repair, heat shock proteins also have a role in the suppression of apoptosis (Simon et al 1995). Hence, the role of NSAIDs as radioprotectors could partly be attributed to their ability to induce heat shock proteins (Simon et al 1995).

Anti-angiogenesis

Angiogenesis is a process by which new vessels grow from established ones. Antigenic factors, such as vascular endothelial growth factor (VEGF), have been associated with the development and maintenance of bone tissue (Harada et al 1994) and pathological processes such as tumour development and rheumatoid arthritis (Brown et al 1993; Ben-Av et al 1995). Recently, it has been suggested that NSAIDs also inhibit cellular proliferation, possibly by an anti-angiogenesis mechanism (Skopinska-Rozewska et al 1998; Pai et al 2000). In the latter study, it was demonstrated that indometacin significantly reduces endothelial cell proliferation (Pai et al 2000). Accordingly, prostaglandins such as PGE_1 and PGE_2 induce production of VEGF (Harada et al 1994; Ben-Av et al 1995), which stimulates angiogenesis. This inhibition of COX enzymes by NSAIDs will certainly lead to anti-angiogenesis.

Uncoupling oxidative phosphorylation

NSAIDs have been commonly associated with gastrointestinal and renal side effects (Mingatto et al 1996; Bamba et al 1998). It was observed that NSAIDs inhibit or uncouple oxidative phosphorylation in mitochondria,

depressing the rate of ATP production, leading to drug-induced cytotoxicity (Mingatto et al 1996).

In-vivo radioprotection by NSAIDs

There is some evidence suggesting that NSAIDs may protect normal tissues from radiation injury in-vivo. It has been found that indometacin provides radioprotection to the haematopoietic system in a mouse model where an overall improvement in the therapeutic ratio of radiotherapy has been determined (Furuta et al 1988). More specifically, indometacin has been found to increase endogenous spleen colony formation after irradiation (Fedorocko & Mackova 1996a, b). In similar studies, nordihydroguaiaretic acid, a compound that inhibits both prostaglandin and leukotriene biosynthesis, also improves post irradiation recovery (Kozubik et al 1993), although esculetin, a compound which inhibits only leukotriene synthesis, does not (Kozubik et al 1994).

There have also been reports that indometacin and aspirin afforded significant radioprotection to the parotid glands in rats during x-ray irradiation (Goldberg 1986). Furthermore, indometacin provided significant radioprotection to the oesophagus in opossum (an animal model selected because the radiation-induced oesophagitis mimics the condition seen in man) thus minimising the development of oesophagitis, which is a major limiting side effect of radiation therapy (Northway et al 1980).

A small-scale double-blind placebo prospective study that combined indometacin (administered orally 25 mg four times a day) and radiotherapy (300 R/day) in the treatment of advanced cancer of the head and neck showed no influence of indometacin on patient survival during a two-year observation period after radiotherapy (Pillsbury et al 1986). However, the study showed a significant protection by indometacin against mucositis. It would appear that using both NSAIDs and radiation offers significant therapeutic gain, whereby NSAIDs provide not only radioprotection to normal tissues, but also offer additive anti-tumour effects (Pillsbury et al 1986; Tonelli et al 2000).

Do NSAIDs have a role as radioprotectors?

Apparently, cells have a myriad of adaptive or homoeostatic mechanisms to prevent, repair and recover from an injury. Inhibition of COX enzymes by NSAIDs reveals only part of their pharmacological effects as therapeutic agents.

The regulation of apoptosis plays a significant role in determining the state of cells and tissue, and NSAIDs certainly have their contributory role. NSAIDs' regulation of apoptosis may be dependent upon, or independent of, COX inhibition. Besides their disruption of the arachidonic acid cascade leading to a decrease in prostaglandin production (Smith 1989; Kontogiorgis & Hadjipavlou-Litina 2002), they can also minimise the generation of reactive oxygen species which are known to be damaging to cells

(Kontogiorgis & Hadjipavlou-Litina 2002). A number of studies on cultured cells have indicated that biological end-points such as transformation, chromosome breakage, sister chromatid exchanges or growth stimulation are modified by SOD (Birnboim & Kanabus-Kaminska 1985).

The uncoupling of the oxidative respiratory chain by NSAIDs induces cytotoxicity (Mingatto et al 1996; Uyemura et al 1997) by increasing the production of superoxide radicals (Gonzalez-Flecha & Demple 2000). However, NSAIDs may provide an antioxidant effect (Birnboim 1982; Wasil et al 1987; Nivsarkar 2000).

The clinical significance of NSAIDs inducing anti-angiogenesis remains to be determined; possibly, poor vascular perfusion leading to necrosis may limit the tumour growth and also arrest the spread of radiation-induced damage (Brown et al 1993).

NSAIDs' downstream effects on the cytokines results in growth inhibition in cells as well as the activation of heat shock protein, which has been demonstrated to have a radioprotective effect.

The ability of NSAIDs to cause cell cycle arrest at G0 or G1 phase limits the number of potentially radiosensitive cells in the mitotic (M) and synthetic (S) phases from damage by radiation, and thus confers radioprotection to normal tissues (Bayer & Beaven 1979; Furuta et al 1988; Morris 1996). Therefore, it might be theoretically logical to consider dosing NSAIDs (be it topical or oral) before radiation.

Prostaglandins

Prostaglandins are the metabolites produced from the arachidonic acid cascade. They are classed as autocoids because of their short half-life and because they exert their effects locally (Smith 1989). Expression of COX enzymes is necessary for their synthesis.

Both COX-1 and COX-2 synthesise the same series of prostanoids. However, Matsumoto et al (1997) have demonstrated that the profile of prostaglandins produced from exogenous arachidonic acid by unstimulated macrophages differs from that of those produced by LPS (lipopolysaccharide)-stimulated cells. The unstimulated cells, which expressed COX-1 but not COX-2, produced thromboxane B_2 (TXB$_2$) > prostaglandin D_2 (PGD$_2$) > PGE$_2$. Cells stimulated with LPS exhibited marked increase in conversion to PGE$_2$ which paralleled COX-2 induction with minimal change in conversion to TXB$_2$ and PGD$_2$. Their studies also showed that formation of PGE$_2$ was mediated predominantly by COX-2, PGD$_2$ by COX-1 and TXB$_2$ by both COX-1 and COX-2.

Levels of eicosanoids in cancer and normal cells

Altered levels of PGE$_2$ and PGI$_2$ have been found in colon cancer as compared with normal tissue distant from tumour (Rigas et al 1993). The level of PGE$_2$ is increased in colon cancer as compared with normal distant colonic tissue, whereas the corresponding level of PGI$_2$ is decreased.

Meanwhile, the levels of PGF$_{2\alpha}$, TXB$_2$ and leukotrienes (LTB$_4$) are not significantly different between cancer and normal tissue. Apparently, in colon cancer tissues, the balance between PGI$_2$ and thromboxane A$_2$ (TXA$_2$) is shifted in favour of TXA$_2$ as compared with normal colonic tissue (Honn et al 1983). The increase in platelet aggregation may facilitate tumour metastasis, presumably at the level of tumour cell interaction with platelets and the vascular wall (Honn et al 1983).

PGEs and angiogenesis

Recent studies have shown that PGE$_2$ activates angiogenesis, which increases the supply of oxygen and nutrients essential for cellular survival and growth (Brown et al 1993; Harada et al 1994; Ben-Av et al 1995; Tonnesen et al 2000). PGE$_2$, on binding to a G-coupled protein prostaglandin receptor subtype 2 (EP2), elevates cyclic $3',5'$-adenosine monophosphate (cAMP) which induces the expression of VEGF that is capable of activating angiogenesis (Ben-Av et al 1995). The ability of PGE$_2$ to trigger angiogenesis may partly explain the cytoprotection observed when misoprostol, a PGE$_1$ analogue, was used in a prospective study to therapeutically negate the effects of radiation injury to normal tissue (Hanson et al 1997; Khan et al 2000). After all, it was proposed quite early that late radiation effects occur as a result of disruption to the blood supply to tissues (Baker & Krochak 1989).

PGE$_2$ – A mediator or modulator of inflammation?

It has been shown that PGE$_2$, released in high amounts by macrophages in inflamed tissues, may exert a feed-forward effect on the expression of its own synthesising enzyme, COX-2 (Hinz et al 2000).

PGE$_2$ displays stimulatory, as well as suppressive, effects on cytokines in a dose-dependent manner (Renz et al 1988). Cytokines, which are mediators that aid intercellular communication (Borish & Rosenwasser 1996), have been shown to influence the expression of COX enzymes (Bishop-Bailey et al 1998; Diaz et al 1998) and accordingly regulate the levels of PGE$_2$. Pro-inflammatory cytokines such as IL-1β and TNF-α selectively induced the transcription of COX-2 gene, whereas TGF-β1, involved in radiation-induced skin lesions (Martin et al 1997), increased the level of both COX-1 and COX-2 enzymes. It was found that PGE$_2$ at low concentration stimulated the production of IL-1β and TNF-α whereas at a high enough concentration suppresses IL-1β and TNF-α production (Knudsen et al 1986; Renz et al 1988; Katsuyama et al 1998).

The fact that PGE$_2$ may function as a modulator, particularly through its feed-forward effect, enhances cellular survival (Rigas et al 1993; Bamba et al 2000) and may possibly explain the lower observed incidence of mouth ulcers and proctitis (an inflammation of rectal mucosa causing rectal bleeding and passage of mucus) (Hanson et al 1997; Khan et al 2000).

PGE$_2$ was observed to maintain not only the cell cytoskeleton but also the mucosal barrier in intestinal cells

(Gerstle et al 1994; Banan et al 2000), contributing to cytoprotection.

Prostaglandins and radioprotection

It has been demonstrated that administration of PGE_1 before a single dose of x-irradiation increases the survival of cells in-vitro (Prasad 1972) and suggested that this was mediated by the prostaglandin-induced increase in the second messenger cAMP (Prasad 1972; Hinz et al 2000).

Recent work (Coleman et al 1994; Takeuchi et al 1994; Burkey & Regan 1995) has established that there are at least four prostaglandin E (PGE) receptors (EP1–EP4). Each PGE receptor differs in its signal transduction mechanisms. These receptor-coupled cascades could probably account for PGE_2's dual functions as pro-inflammatory and anti-inflammatory (Knudsen et al 1986; Renz et al 1988; Strassmann et al 1994; Kambayashi et al 1995). It has been demonstrated that PGE_2, on binding to EP2 and EP4 receptors, inhibits macrophage functions probably by suppressing the production of pro-inflammatory cytokines such as TNF-α and IL-1β (Katsuyama et al 1998).

In later studies, prostaglandin-induced radiation protection in-vivo has consistently been found (Hanson & Thomas 1983; Hanson 1987; Hanson & DeLaurentiis 1987; Walden et al 1987; Hanson et al 1988). In all these studies, the findings can be summarised such that arachidonic acid at a dose of 1 mg per 30 g mouse increased intestinal clonogenic cell survival at 15.0 Gy (Hanson et al 1988) but at 500 μg per mouse or lower doses, arachidonic acid did not provide radioprotection. The natural prostaglandins protected intestinal clonogenic cell survival ranging from about 150% above controls for PGA_2 up to 325% above controls for PGI_2 (Hanson et al 1988). The PGE_1 analogue misoprostol protected to a greater degree, increasing intestinal clonogenic cell survival to about 600% of controls. This PGE_1-induced protection may not be directly associated with DNA strand breaks, but with increased synthesis and release of PGE_2 (Hanson 1987; Mercer et al 1996).

Prospective studies in man

Clinical studies have suggested that the prostaglandin analogue misoprostol can prevent radiation-induced side effects (Hanson et al 1997; Khan et al 2000). It has been shown that patients rinsing their mouth with misoprostol solution before undergoing radiotherapy on head and neck cancers had a reduced incidence of mouth ulcers (Hanson et al 1997). Similarly it has been shown that prostate cancer patients who used a 400-μg misoprostol rectal suppository one hour before radiation had a reduced incidence of radiation-induced proctitis (Khan et al 2000).

However, other prospective studies showed no significant benefit of misoprostol in preventing bone-marrow-transplant- or chemotherapy-induced mucositis (Labar et al 1993; Duenas-Gonzalez et al 1996). Authors of both studies suggested the high incidence of herpes simplex virus (HSV) infection as a possible reason, although the possibility of insufficient concentration of misoprostol should also be considered.

Do prostaglandins have a role as radioprotectors?

At this juncture, we know for certain that activator protein-1 (AP-1) and TGF-β1 gene expression are co-activated on radiation and may be responsible for the skin lesions observed (Martin et al 1997). AP-1 is a collective name for a class of transcription factors implicated in the regulation of many cellular processes such as proliferation, differentiation and apoptosis. Both AP-1 and TGF-β1 activity will undoubtedly contribute partially to the regulation of PGE_2 levels, which in turn has a modulatory effect on regulating the levels of pro-inflammatory cytokines (Knudsen et al 1986; Renz et al 1988; Katsuyama et al 1998).

Whether the prostaglandin levels provide radioprotection to the cells by maintaining cytoskeletal integrity, activation of angiogenesis or enhancing cellular proliferation (or a combination of these) remains an area to be extensively studied.

Conclusions

Both NSAIDs and prostaglandins show great potential in minimising the adverse effects of radiotherapy on normal tissue. The notion of dosing both NSAIDs and prostaglandin (misoprostol) together before radiotherapy may offer synergistic effects towards radioprotection on normal tissue.

It has been proposed that a decrease in cell cycle time for proliferating cells and the recruitment of stem cells from resting G0 state into actively cycling state can shorten the recovery time from the acute effects of radiation (Morris 1996). NSAIDs are capable of cell cycle arrest by shifting the cell cycle towards a quiescence state G0/G1 (Hanif et al 1996; Pai et al 2000). It is also evident that cells in M and S phases of the cell cycle are more radiosensitive than those in the G0/G1 (Morris 1996). Therefore, will pre-treatment with NSAIDs minimise the proportion of cells being damaged by radiation and will their subsequent removal decrease the cell cycle time allowing a shorter cell population doubling time, inducing a rapid recovery? After all, it has been shown that, on removal of NSAIDs, the arrested cells would resume growth with considerable degree of synchrony (Bayer et al 1979). Furthermore, the use of NSAIDs does not interrupt the radiation therapy, but instead enhances the radiotherapeutic ratio (Pillsbury et al 1986; Palayoor et al 1998; Tonelli et al 2000).

It is apparent that NSAIDs have radioprotection towards cells with a rapid turn-over, such as the mucosa (Pillsbury et al 1986). Their radioprotection towards slow turn-over parenchymal cells (cells that attribute to late radiation effects) has yet to be elucidated.

NSAIDs have been shown to exhibit antioxidant effect (Nivsarkar 2000; Wasil et al 1987). Radiation causes

normal tissue damage by bystander effects (Grosovsky et al 1996; Wu et al 1999; Zhou et al 2000). Potentially, NSAIDs may limit radiation injury by reducing this bystander effect. Activation of protein kinase C mediates radioprotection in cells by an anti-apoptotic mechanism (Fuks et al 1994; Haimovitz-Friedman et al 1994; Gamble et al 2000). NSAIDs trigger apoptosis (Chan et al 1998; Thompson et al 2000). Whether NSAIDs would intervene or associate with the action of protein kinase C is questionable and requires further study.

NSAIDs can activate heat shock proteins, which have the ability to protect protein moieties from radiation-induced damage (Simon et al 1995; Soncin & Calderwood 1996; Housby et al 1999).

Prostaglandin E$_2$ at a sufficiently high concentration inhibits TNF (tumour necrosis factor)-α (Renz et al 1988; Thompson et al 2000) and increases cellular proliferation (Rigas et al 1993). The use of misoprostol, a PGE$_1$ analogue, mimics the action of PGE$_2$ at high concentrations (Hanson et al 1988). Is it possible that the radioprotective mechanism afforded by misoprostol, which is administered before irradiation, elevates the normal cell population sufficiently to meet that fraction of cells that will be destroyed by the radiation dose, thereby ultimately conferring an overall protection to the normal tissue?

Because PGE$_2$ is found in high levels in tumour cells (Rigas et al 1993) and enhances cellular survival (Brown et al 1993; Ben-Av et al 1995), it might be appropriate to suggest that misoprostol should not be administered systemically unless a targeted approach to protect only the normal tissue is made available. Thus, the use of misoprostol should be confined to local application (topically).

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