REVIEW

Therapeutic implications of disorders of cell death signalling: membranes, micro-environment, and eicosanoid and docosanoid metabolism

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Disruptions of cell death signalling occur in pathological processes, such as cancer and degenerative disease. Increased knowledge of cell death signalling has opened new areas of therapeutic research, and identifying key mediators of cell death has become increasingly important. Early triggering events in cell death may provide potential therapeutic targets, whereas agents affecting later signals may be more palliative in nature. A group of primary mediators are derivatives of the highly unsaturated fatty acids (HUFAs), particularly oxygenated metabolites such as prostaglandins. HUFAs, esterified in cell membranes, act as critical signalling molecules in many pathological processes. Currently, agents affecting HUFA metabolism are widely prescribed in diseases involving disordered cell death signalling. However, partly due to rapid metabolism, their role in cell death signalling pathways is poorly characterized. Recently, HUFA-derived mediators, the resolvins/protectins and endocannabinoids, have added opportunities to target selective signals and pathways. This review will focus on the control of cell death by HUFA, eicosanoid (C20 fatty acid metabolites) and docosanoid (C22 metabolites), HUFA-derived lipid mediators, signalling elements in the micro-environment and their potential therapeutic applications. Further therapeutic approaches will involve cell and molecular biology, the multiple hit theory of disease progression and analysis of system plasticity. Advances in the cell biology of eicosanoid and docosanoid metabolism, together with structure/function analysis of HUFA-derived mediators, will be useful in developing therapeutic agents in pathologies characterized by alterations in cell death signalling.

Abbreviations

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NSAID, nonsteroidal anti-inflammatory drug; PG, prostaglandin; AA, arachidonic acid; HUFA, highly unsaturated fatty acids with 4 or more bonds, for example, arachidonic, eicosapentaenoic and docosahexaenoic acids (4, 5 and 6 double bonds respectively); PUFA, polyunsaturated fatty acids, with 2 or more unsaturated C-C bonds; HUFA, highly unsaturated C20 fatty acid, with 3 or more unsaturated C-C bonds

Many therapeutic agents influence cell death signalling and highly unsaturated fatty acid (HUFA) metabolism (Figure 1). These agents may act at the level of metabolic events affecting apoptosis, enzyme systems and cofactors, agents affecting cell cycle progression and DNA repair, and oncogene expression. Intracellularly, agents affecting organelles and the

mitochondrial intrinsic pathway, endoplasmic reticulum-associated stress pathways and lysosomal autophagy can have profound effects on cell death. There has also been development of agents affecting transcellular signalling via the extrinsic pathway, oxidative stress, growth factors and lipid mediators, ion and metabolite flux, adhesion and migration.

Figure 1

Cell death regulation: Signalling pathways and potential sites for pharmacological intervention. Pathways promoting cell death via extrinsic and intrinsic signalling are shown to the left, and cytoprotective pathways, to the right, although the outcome of signalling depends on signal intensity, frequency and other signals, and the micro-environment. Red arrows indicate sites of pharmaceutical intervention, for example, agonists and antagonists of prostaglandin receptors (PGRs), antagonists of Ca²⁺ transport (Ca²⁺ antag), growth factor (GF) inhibitors and lipoxygenase inhibitors (LO inhib). Both intrinsic and extrinsic pathways are activated by HUFA release and inhibited by PGs of E, F and D series via PGR signalling to PKC and cAMP. Reactive oxygen intermediates (ROS) may activate stress kinases, including extracellular regulated protein kinase (ERK). ROS may elicit DNA damage, whose repair is controlled by sensors and repair processes involving p53, acetylation/ubiquitylation and autophagy, controlled by autophagy genes and mTOR kinase. These pathways are inhibited by lysosome stabilizers (Lys inhib). Cytoplasmic phospholipase A₂ (cPLA₂) also activates NF-κB, and inhibits cell cycle progression via cyclin-dependent kinase 4 (CDK4) and cyclin D1. ROS may also activate the intrinsic pathway by depolarizing the mitochondrial membrane (σ_ψm), leading to cytochrome release and apoptosis, via caspases 9 and 3. Protein phosphatase 2A (PP2A) plays a role in gene expression, cell division and signal transduction. PP2A is activated by ceramide, produced by hydrolysis of membrane sphingomyelin (SM) in response to stress-related stimuli. The cellular response to hypoxia is coordinated by hypoxia-inducible factor 1α (HIF-1α), in tumour cells this leads to up-regulation of angiogenic factors such as vascular endothelial growth factor. Disorders of HUFA metabolism have been identified in mitochondrial carnitine palmitoyl transferase (CPT), which transport HUFA into mitochondria. Sphingosine-1-phosphate (S1P) enhances survival by signalling cell adhesion in colon cancer and certain leukaemias. Transcellular signalling is shown, as PGs and their anandamide derivatives, lipoxygenase products (LT, HETE) and GFs released from activated cells, together with shorter range mediators such as Ca²⁺, NO, AA and ROS, all of which may act as micro-environmental factors controlling cell death.

Also, recently there has been an expansion in agents affecting physiological systems, including angiogenesis, immune surveillance, and development and differentiation. These signals will be discussed, together with questions about lipid factors

that lead to the decision to activate cell death or survival (Leaver and Hardingham, 2010; Wyllie, 2010). Topical issues in cell death signalling and how this signalling can be influenced by therapeutic agents will be discussed (Table 1). It will

be argued that membrane responses and membrane-associated mediators linked to HUFA play a key role in the pathophysiology of cell death.

HUFA responses to cell death signals are of vital importance in the pharmacology of some of the most complex and intractable diseases (Lukiw and Bazan, 2010). They are a primary component of cell membranes, which create cellular compartments and micro-environments, and HUFA-derived lipid mediators participate in communication between compartments. The identity of many HUFA-derived mediators is known, but the flux of mediators and micro-environmental signals controlling cell death are poorly defined at cell and systems level. Detailed analysis of the pathology of cell death signalling is being used to identify key cellular signals and agents that modulate their activity. Additionally, complex polyunsaturated fatty acid (PUFA) derivatives, for example, conjugated linoleic acids (CLAs), influence cellular metabolism, cell viability and the survival of cancer cells. These CLAs have been comprehensively reviewed (see Wahle *et al.*, 2004). In the first part of this review, developments in signalling will be outlined which are leading to potential sites of therapeutic intervention. This will be followed by specific examples of HUFA-derived mediators, whose impact on cell survival is becoming better characterized in pharmacological terms.

The pathophysiology of cell death signalling

Recent advances in cell death signalling have led to a deeper understanding of the networks and systems associated with

cell pathology (Figure 1). This has been important in developing therapies in complex multifactorial diseases, such as cancer and degenerative disease (Galluzzi *et al.*, 2007). New system-based approaches to drug development, such as targeting multiple genes, and transcriptional and environmental components, are being used in diseases associated with cell death signalling (Chawla *et al.*, 2001; Kirkegaard *et al.*, 2005; Psaila and Bussel, 2008; Rader and Daugherty, 2008; Miller *et al.*, 2009; Uttara *et al.*, 2009; Wang *et al.*, 2009; Nair *et al.*, 2010; Meuillet, 2011; Prakobwong *et al.*, 2011). Advances in stem cell biology have also helped to characterize cell types important in regenerative and degenerative processes (Hardingham *et al.*, 2010; Nair *et al.*, 2010). In many cases, these approaches are in the early stages of development. However, in these systems, it is crucial to disentangle causative events and reactive changes, and to identify key events and signals, in order to develop therapeutic agents active in cell death signalling pathways.

Cell death signalling pathways

Cell death is executed by a complex and sophisticated signalling network, with multiple effectors and mediators, crosstalk, overlapping signalling pathways and diverse end points (Figure 1; Vicencio *et al.*, 2008). In this review, signalling by lipid mediators at membrane level, intracellular compartmentalization and the role of HUFA in transmitting micro-environmental signals to cell death signalling within the cell will be discussed (Table 1).

Several evolutionarily conserved proteins protect against cell death, including Bcl-2, which regulates the intrinsic mitochondrial pathway of cell death, and p53, which is associated with genomic integrity checkpoints (Figure 1). Many

Table 1

Topical issues in cell death signalling and HUFA metabolism

1. **Cellular organization of cell death signalling** Relative roles of mitochondria, endoplasmic reticulum and plasma membrane in key decisions in cell death signalling (Bröker *et al.*, 2005; Alonso *et al.*, 2008; Pellicano *et al.*, 2009; Wu *et al.*, 2009b; Benadiba *et al.*, 2010; Wyllie, 2010). (pharmacological relevance includes new targets for drug development, agonists and antagonists, reversibility)
2. **Cell-cell communication and the micro-environment** Role in angiogenesis, oncogenesis, degenerative signalling (Adibhatla and Hatcher, 2006; Combrinck *et al.*, 2006; Rizzo and Leaver, 2010) (pharmacological relevance: how can this be modified?)
3. **Systems communication** Relative contribution of vascular signalling, tissue signals, cell/organ plasticity (Rush *et al.*, 2007; Miller *et al.*, 2009; Pertwee, 2009; 2010; Ahmad *et al.*, 2010; Sun *et al.*, 2010). [bioengineering of therapeutics, localization and targeting cellular membranes, vasculature]
4. **Role of membrane HUFA** (Bisogno *et al.*, 1999; Akiba *et al.*, 2000; Rizzo *et al.*, 2002; Bhatena, 2006; Appendino *et al.*, 2009; Brown *et al.*, 2010; 2011; Lukiw and Bazan, 2010; Picq *et al.*, 2010). (pharmacological/chemical/dietary modification)
5. **Stress signalling** Pro-apoptotic activities of HUFA via stress signalling pathways: pathophysiological role. Mediators and signalling pathways (Adibhatla and Hatcher, 2006; Ito *et al.*, 2006; Carrasco *et al.*, 2007; 2008; Chen and Chang, 2009; Xue *et al.*, 2009; Bidwell *et al.*, 2010). (agonists and antagonists of HUFA, stress kinases, mediators including NF κ B, HIF1)
6. **Cell survival signalling** Cytoprotective activities. Role of PGE₂, PGD₂, 15d-PGJ₂ and their associated receptors, alternative signalling via PPAR, Bcl, endocannabinoid and resolvin pathways (Payner *et al.*, 2006; George *et al.*, 2007; Andrade da Costa *et al.*, 2009; Carlson *et al.*, 2009; Bai *et al.*, 2010) (agonists and antagonists, systems specificity)
7. **Potential of molecular genetics and stem cell developments** (Rader and Daugherty, 2008; Hardingham *et al.*, 2010) (pharmacological advances in diagnosis, epidemiology, bioengineering)
8. **Multiple hits model of cell death** Combination therapy of agents affecting different cell death signals to avoid escape via overlapping multifunctional pathways (Alonso *et al.*, 2008; Lasithiotakis *et al.*, 2008; Psaila and Bussel, 2008) (multi-system and agent approaches, especially in diseases with complex aetiology such as degenerative and oncogenic disease)

Therapeutic implications are shown in parentheses ().

Table 2

Organ and species specific HUFA distribution

Molecular HUFA distribution

Membrane phospholipids 2-acyl position of PtdCho, PtdEt (main PLA₂ substrates), PtdSer, PtdIns (main PLC substrate), alkyl phospholipids, endocannabinoids, sphingolipids

Intracellular and secreted lipids HUFA in free fatty acids (released following PLA₂, PLC and DAG lipase activation); COX, LOX and cyt P450 metabolites, resolvins, endocannabinoid metabolites, PAF, triacylglycerols and glycerides, cholesterol esters

Subcellular HUFA distribution

Plasma membrane, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosome, proteasome, secretory granules, apoptosome, peroxisome. (Distinct uptake, composition and metabolism in different membrane systems, leading to signalling compartmentation and lipid micro-environments)

Tissues and organ systems associated with HUFA metabolism

Liver (precursor chain elongation, desaturation, release of HUFA, metabolism)

Blood cells: RBC, platelets, leucocytes, plasma albumin (HUFA transport)

Vasculature endothelial cells (HUFA uptake, especially to brain)

Lung (HUFA uptake and metabolism)

Brain, CNS, retina (HUFA uptake, specific n-3 functions)

GI system (HUFA and precursor uptake, PG sensitive)

Immune system (HUFA release, LT sensitive)

Species

Mammalian (specific eicosanoid, docosanoid metabolism and activities, paracrine actions)

Fish (higher n-3/n-6 HUFA composition)

Plant (precursors of both n-6 and n-3 HUFA)

HUFA levels vary in different lipid classes, dependent on diet and pathophysiological stimuli. The cell membrane phospholipids phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEt) generally contain the highest available cellular HUFA stores, released in response to activation of phospholipase A₂ (PLA₂). HUFA in phosphatidylinositol (PtdIns) is released by PLC and DAG/monoacylglycerol (MAG) lipase activation. Released HUFAs are rapidly metabolized by COX to form PG, lipoxygenase (LO) to form leukotriene (LT) and cytochrome P450 (cyt P450) enzymes, or are re-esterified. Resolvins and endocannabinoids and platelet activating factor (PAF) may use HUFA pathways of metabolism, but also have distinct synthetase pathways and receptors. HUFA are present in all subcellular membranes, although phospholipid and HUFA distribution is membrane specific. Tissue and species HUFA levels are also specific, and depend on supply and demand, and specific functions, for example, neurotransmission or inflammatory processes require selective uptake and release, and both processes are often compromised in degenerative disease.

key genes associated with cell death exert other vital functions associated with survival. Indeed, it has been postulated that no specific 'cell death' genes exist, only genetic and epigenetic elements that control cell survival under certain circumstances. Thus, mediators, metabolites, signalling systems and organelles such as mitochondria are involved in the pathophysiology of cell death as well as other physiological functions. This has implications in therapeutics, where partial agonist and antagonists may be important in order to preserve physiological functions, while targeting pathological changes with overlapping pathways and mediators.

The characteristics of cell death are diverse: necrosis, apoptosis and autophagy may be different and distinct modes of cell death, although many pathophysiological processes exhibit characteristics of multiple modes of cell death (Kroemer *et al.*, 2009). Thus, the catastrophic stress and necrosis of vascular stroke differ from slower degenerative changes in vascular disease. Yet, both processes use overlapping pathways and mediators, for example, endothelial cells responding to death signals such as hypoxia and stress signals via the intrinsic pathway (Figure 1, Rizzo and Leaver, 2010). A

further cell death pathway involving lysosomes has been identified. Recent studies on lysosomal membrane metabolism have implicated lysosomes in autophagy, and have led to development of agents that affect lysosomal stability (Alonso *et al.*, 2008; Galluzzi *et al.*, 2008; Geng *et al.*, 2010). A fruitful field of drug development has focused on early signalling elements, such as agents acting on protein kinases (Bain *et al.*, 2007).

Triggers of cell death may include physical or chemical insult, and hormonal and other cell- and system-derived signals, activating various cellular mediators. The transduction pathways of cell death are diverse (Vicencio *et al.*, 2008; Harr and Distelhorst, 2010) involving membrane systems, including the plasma membrane, intracellular membranes and organelles, and membrane-derived lipid mediators with nuclear and transcriptional actions (Table 1, Colquhoun, 2010; Leaver *et al.*, 2010; Cui *et al.*, 2011; Rizzo, 2011). A characteristic of eukaryotic plasma and intracellular membranes is their high PUFA content (Table 2). PUFAs may be released from membranes in response to pathophysiological stimuli, and either exert a direct action, or be metabolized by

lipoygenase or COX to mediators with pathophysiological activities (Figure 2). These mediators have a short half-life and physical range, being limited to intracellular compartments in the case of free radicals, and highly reactive lipid peroxides, or having transcellular and local systemic activity in the case of PGE₂. Lipid mediator synthesis may be influenced by micro-environmental factors (Nair *et al.*, 2010), and pharmacological agents such as aspirin may result in the synthesis of novel anti-inflammatory mediators (Lukiw and Bazan, 2010; Serhan and Haeggstrom, 2010).

PUFA release under pathological conditions

The HUFA cascade

Mediators and key regulatory points of the cell death cascade are shown in Figure 1. Pathways of arachidonic acid (AA) release and metabolism are shown, although n-3 HUFA (Figure 2) may play a role in certain tissues and species (Table 2). HUFA release is initiated by phospholipase activation. Phospholipases A₂, C and D are activated in response to cell surface ligand binding, intracellular calcium mobilization and activation of cell stress signals (Figure 1). The type and amount of released lipid mediators depend on the stimulus, cell type, nutritional and metabolic state, and membrane composition (Table 2). The release of fatty acids can also be regarded as physiological when the actions of lipases are constitutive (require no external stimulus) or occur in response to hormones, for example, vascular cell release of AA in response to vasopressin (Liu and Taylor, 2006), which is a calcium-dependent response. There is not always a requirement for increased intracellular calcium to activate phospholipases, indeed in monocytes both processes can occur in parallel when both calcium-dependent and calcium-independent release of AA may elicit increased eicosanoid formation (Hoffman *et al.*, 1988). HUFA signalling influences early events in two interacting pathways of cell death, intrinsic and extrinsic pathways (Figure 1). The intrinsic pathway, activated by stress signals, involves mitochondrial factors and Bcl family members, while extrinsic signalling is initiated by cell surface receptors of the TNF family and extrinsic signals. PUFA/ HUFA release may occur at the plasma membrane, or at intracellular membranes, such as endoplasmic reticulum and mitochondrial membranes. AA and other PUFA may exert direct effects on stress signalling factors and genes (Rizzo *et al.*, 1999; 2002). AA regulates gene expression directly via p38 MAPK, ERK and JNK, increasing transcription of AP-1-containing genes. These events are inhibited by tyrosine kinase inhibitors. These signalling systems present potential therapeutic targets, and the opportunity for specifically targeting pathological pathways, while protecting physiologically important signals, such as basal COX activity essential for gastric integrity, endothelial and vascular protection, or brain specific signalling via n-3 HUFA-associated pathways.

Pathology of PUFA release

PUFA released in response to stress or TNFR signalling may be oxidized by lipoperoxidation (Figure 2) to reactive oxygen

species (ROS), which rapidly depolarize mitochondria, leading to cytochrome c release, apoptosis inducing factor release and cell death (Figure 1). ROS may be generated intracellularly or extracellularly in response to ionizing radiation, stress signals, hypoxia/reperfusion, mitochondrial uncoupling, free radical generation, or from NO or HUFA peroxidation, to activate stress kinases, including p38 MAPK or JNK (Ito *et al.*, 2006; Chen and Chang, 2009; Uttara *et al.*, 2009). ROS may also exert genotoxic activity, activating endonuclease and ceramide cell stress signalling (Miller *et al.*, 2009). These pathways may be exaggerated, for instance, in tumours over-expressing Akt, a key apoptotic signal sensitive to ROS (Kirkegaard *et al.*, 2005; Meuillet, 2011). Also, pathological changes in the ceramide stress pathway, affecting sensitivity to chemotherapy and radiotherapy, have been detected (Orgertman and Hannun, 2004). HUFA-derived ROS may also be formed directly within membrane phospholipids, but these appear to have similar pro-apoptotic activities via stress signalling pathways (Deigner and Hermetter, 2008).

Pathological control over PUFA release and metabolism may be exerted at the level of phospholipase activation, for example, sPLA₂ and cPLA₂ stimulate tumour cell migration and proliferation (Rizzo *et al.*, 2002; Denizot *et al.*, 2009). Hypoxia during stroke or vascular injury may elicit cell death via ROS-dependent activation of apoptosis (Adibhatla and Hatcher, 2006). PUFA and associated ROS activity are limited by rapid re-esterification pathways, which are also important in membrane remodelling (Farooqui and Horrocks, 2006).

Selective intracellular uptake of PUFA is critical, and disorders of PUFA uptake have been identified, for example, mitochondrial carnitine palmitoyl transferase, involved in transport of HUFA into mitochondria, which is inhibited by PGE₂ (Colquhoun, 2010). Additionally, as shown in Figure 1, PUFA and their metabolites can act as transcellular mediators in both activation of and protection from cell death signals. This notion emphasizes a critical role of lipid mediators in influencing the micro-environment, and creating conditions for generation of apoptotic or anti-apoptotic signals (Greenhough *et al.*, 2009). Thus, the decision of cells to survive or undergo death is influenced by PUFA and their metabolites in the micro-environment.

Anti-apoptotic survival pathways involving HUFA are relevant in pathologies characterized by increased angiogenesis, where HUFA-derived eicosanoids, such as PGE₂, may play a critical role in affecting endothelial cell angiogenic responses, and release of angiogenic growth factors from tumour cells (Baryawno *et al.*, 2008; Bai *et al.*, 2010; Rizzo, 2011).

Therapeutic aspects of cell death signalling

Topical issues in therapeutics

The inappropriate regulation of cell death has been implicated in many pathological processes, ranging from cancer to vascular disease (Galluzzi *et al.*, 2007). There is demand for drugs that selectively induce cell death (e.g. in neoplastic cells) or agents that antagonize or attenuate it (in degenerative disease or ischaemia). Increasing numbers of therapeutic agents act on cell death signalling pathways (Figure 1 and

Figure 2

Pathways of PUFA release and metabolism. PUFA released from cell membranes in response to pathophysiological stimuli either exerts a direct action localized within the cell, or may be metabolized by lipoperoxidation or COX to mediators with a wider range of activity and capacity to act on cells, organs, tissues, and exert pathophysiological activities (endothelial/cancer cell migration, proliferation, adhesion). Selectivity lies at the level of uptake, transport, esterification/lipase cycles and phospholipase. There may also be specificity at the level of n-3 and n-6 series metabolism. PGs are synthesized from essential fatty acids derived from diet, predominantly esterified to complex lipids. Three primary PG precursor PUFAs are dihomogammalinolenic acid (DGLA, cis-5,8,11-eicosatrienoic acid; 20:3 n-6), AA (cis-5, 8, 11, 14 eicosatetraenoic acid; 20:4 n-6) and EPA (cis-5,8,11,14,17-eicosapentaenoic acid; 20:5 n-3). PUFAs, mainly esterified in the *sn*-2-position of membrane phospholipids, are released via phospholipase A₂ (PLA₂, Akiba *et al.*, 2000) or in a two-step process involving phospholipase C and diglyceride/monoglyceride lipase. Free DGLA, AA and EPA within mammalian cells are metabolized via COX to series 1, 2 and 3 PGs with 1, 2 and 3 double bonds respectively. The most abundant PUFA is AA. AA and EPA are converted to unstable endoperoxide intermediate by COX, and to 2- and 3-series PGs, PGE, PGD and PGF_{2α} by specific PG synthases.

Table 3

HUFA metabolism in pathological conditions, indicating pharmacological targets

Pathology	Pharmacological target	Reference
Age-related degenerative processes	ROS/SOD	Ito <i>et al.</i> , 2006
Aging and stress (retinal ischaemia)	PGE ₂ agonists, EP2R	Osborne <i>et al.</i> , 2009
	Resolvin, PAF	Lukiw and Bazan, 2010
Microglia (neuroprotection)	PGE ₂ agonists, EP1R	Carlson <i>et al.</i> , 2009
Alzheimer's disease	PGE ₂ agonists, EP2R	Combrinck <i>et al.</i> , 2006
Neuroprotection	PGD ₂ agonists, DP1R	Liang <i>et al.</i> , 2005b
Neovascularization in oncology	COX inhibitors	Rizzo, 2011
Endothelial proliferation	Liperoxide, p38, MAPK	Cui <i>et al.</i> , 2011
Invasion, proliferation, survival (Various cancers)	COX inhib (aspirin, NSAID)	Rothwell <i>et al.</i> , 2011
		Elwood <i>et al.</i> , 2009
		Cuzick <i>et al.</i> , 2009
Osteolytic tumour growth	EP2R	Takahashi <i>et al.</i> , 2008
Glioma, leukemia growth	Lysosomal membrane, autophagy	Alonso <i>et al.</i> , 2008
		Vicencio <i>et al.</i> , 2008
Glioma cell growth	mPGE synthase-1, pKAI	Payner <i>et al.</i> , 2006
Anaplastic astrocytoma	PGD ₂ synthase	Payne <i>et al.</i> , 2008
Medulloblastoma growth	EP2	Baryawno <i>et al.</i> , 2008
Colorectal cancer	COX-2 metabolites	Chell <i>et al.</i> , 2006
Colon cancer	Fas ligand EP1R	O'Callaghan <i>et al.</i> , 2008
Esophageal adenocarcinoma	PGE ₂ R antagonists	Piazuelo <i>et al.</i> , 2006
Oral squamous cell carcinoma growth	PGE ₂ , EP3R	Hoshikawa <i>et al.</i> , 2009
CML	Mitochondrial disruption	Pellicano <i>et al.</i> , 2009
	Src tyrosine kinase	Nair <i>et al.</i> , 2010
Endometrial cancer (epithelial cell invasion)	Epithelial PGF _{2α} ADAMTS1	Keightley <i>et al.</i> , 2010
Stroke (neuroprotection)	PPAR-γ	Pereira <i>et al.</i> , 2006
Cerebral infarction	DP1 receptor	Thura <i>et al.</i> , 2009
Brain ischaemia-reperfusion injury	PGD(2) DP1R	Saleem <i>et al.</i> , 2007
	PGE ₂ , EP2R	McCullough <i>et al.</i> , 2004
Neurons oxidative stress (β-amyloid exposure)	EP2 and EP4 agonists	Echeverria <i>et al.</i> , 2005
Pulmonary apoptosis (haemorrhagic shock, sepsis)	PPAR-γ, mito Δψm	Chima <i>et al.</i> , 2010
Gastric epithelial <i>Helicobacter pylori</i> -induced apoptosis	NF-kB, Bcl-2	Chu <i>et al.</i> , 2003

Superoxide dismutase (SOD) inhibitors increased lifespan in rodent models, and inhibitors of reactive oxygen (ROS) generation attenuate degenerative processes involving glutaminergic neurotransmission. Receptors for PGE₂ (EP) and PGD₂ (DP) are cytoprotective in neurodegenerative processes and vascular disease. In endometrial adenocarcinoma cells, PGF_{2α} via the FP receptor regulates disintegrin and metalloproteinase with thrombospondin repeat 1 (ADAMTS1). Microsomal PGE (mPGE) synthase-1 regulates human glioma cell growth via PGE₂-dependent activation of type II protein kinase A (pKAI). Ciglitazone, a novel inhibitor of PPAR, attenuated the intrinsic pathway of apoptosis and mitochondrial membrane potential (Δψm).

Table 3). However, limitations in clinical trials using inhibitors of terminal cell death effectors, the caspases, indicate the importance of selecting early triggering events and mediators, before the cascade leading to cell death becomes irreversible.

Targeting early signals and pathological processes has been the basis of inhibitors of, for example, dual SRC/BCR-Abl kinase inhibition of tumour-initiating cells (Bain *et al.*, 2007; Nair *et al.*, 2010). Also, targeting early events involving mitochondrial disruption is effective in killing chronic myeloid leukemia (CML) progenitor cells (Pellicano *et al.*, 2009). Other

pharmacological agents include those affecting ion flux associated with HUFA release (Harr and Distelhorst, 2010). The role of antioxidants in limiting excessive ROS in inflammatory, hypermetabolic and degenerative disease is also the subject of current research (Uttara *et al.*, 2009). The PPARs are another group of HUFA receptors with up-regulated cell death signalling activity in hypoxia and various pathologies (Table 4).

Angiogenesis is a current area of therapeutic development, targeting vascular endothelial growth receptors and endothelial cell signalling. Endothelial cell growth and

Table 4

Pro- and anti-apoptotic activities of PPAR agonists and antagonists, indicating proposed mediators, and selective actions in tumour, normal tissue and tissue/cells affected by degenerative disorders

PPAR species	Signalling elements	Pathological target*	Reference
α KO	NF- κ B	+ Tumour radiation-induced stress	Zhao <i>et al.</i> , 2007
β	H2O2	– Endothelial cell	Jiang <i>et al.</i> , 2009
β	FA oxidation	– Pancreatic β cell	Wan <i>et al.</i> , 2010
$\beta\delta$	Oedema	– Spinal cord injury	Paterniti <i>et al.</i> , 2010
δ	COX	+ Colon cancer	Wu <i>et al.</i> , 2009a
δ	TGF β	– Vascular smooth muscle	Kim <i>et al.</i> , 2009
γ	Bcl-2	– Oxid stress cardiomyocyte	Ren <i>et al.</i> , 2009
γ	Akt	– Cardiomyocyte	Kilter <i>et al.</i> , 2009
γ	Ψ m, Bcl-2	– Uvb, mitochondria	Wu <i>et al.</i> , 2009b
γ	NO	+ Retinoid assoc tumour	Shankaranarayanan <i>et al.</i> , 2009
γ	Cannabinoid	+ Hep92 apoptosis	Giuliano <i>et al.</i> , 2009
γ	P63 p73	+ Ovarian cancer	Kim <i>et al.</i> , 2011
γ	15LO	+ Non-small cell lung cancer	Mao <i>et al.</i> , 2010
γ	Chromatin	– Growth HL-60 cells	Liu <i>et al.</i> , 2009b
γ	NF- κ B, STAT-	– Growth biliary cancer	Prakobwong <i>et al.</i> , 2011
γ	XIAP, CZG	+ Promyelocytic leukaemia	Liu <i>et al.</i> , 2009a
γ		– Liver in haemorrhagic shock	Zingarelli <i>et al.</i> , 2010
γ	COX-2	– Proliferation, pancreatic cancer	Sun <i>et al.</i> , 2009
γ	C/EBP, p53	+ Hepatic stellate cells	Wang <i>et al.</i> , 2009
γ	NF- κ B, GSK-3 β	– Growth colon cancer	Ban <i>et al.</i> , 2010

* indicates protection against apoptosis, + pro-apoptotic activity, T tumour cells, mito mitochondria. KO, gene knockout study; FA, fatty acid; LO, lipoxygenase.

migration play a key role in angiogenesis (Tables 3 and 5) and are controlled by autocrine and paracrine growth factors and lipid mediators which influence endothelial cell survival (Rizzo and Leaver, 2010). Survival mechanisms may be important in endothelial cell function, where advances in adhesion biology have helped define processes associated with angiogenesis and repair in damaged tissue (Rader and Daugherty, 2008). On the other hand, pro-apoptotic endothelial targeting has recently been the focus of anti-angiogenic therapy in invasive tumours (Lord-Dufour *et al.*, 2009; Rizzo and Leaver, 2010). The role of vasoactive paracrine HUFA-derived signals, such as eicosanoids and docosanoids, is an important area of therapeutic investigation. This will be discussed further, see following sections on the role of prostaglandins (PGs) in control of cell death signalling, and advances in cyclooxygenase pharmacology: receptors and signals that confer protection by preventing cell death (the drug/receptors nomenclature use conforms to the British Journal of Pharmacology's guide to receptors and channels, Alexander *et al.*, 2011).

Additionally, the principle of combined therapy is currently used in selecting targets to evade alternative signalling, for example, in many oncology trials, combinations of agents acting at different targets, for example. Growth factor antagonists, acting via intrinsic and extrinsic apoptotic pathways, are often combined with agents that affect DNA damage

repair, or cell cycle checkpoints. Membrane, mediator and micro-environmental signalling at multiple locations is also relevant to stem cell strategies, where more than one cell type may be involved in pathogenesis.

Targeting n-3 HUFA metabolism

The n-3 essential fatty acids (Figure 2 and Table 2) are currently a focus of interest, because of the ability of n-3 HUFA-based drugs, dietary approaches and nutraceuticals to modify membrane HUFA content. This has arisen because of perceived beneficial cardiovascular effects, but brain targets may also be important. Recent advances in genetics, proteomics and lipidomics have given insights into the substrate specificity of HUFA release (Denizot *et al.*, 2009). Additional approaches have included using naturally occurring n-3 HUFA, development of specific n-3 HUFA-derived agonists and antagonists (Picq *et al.*, 2010), and agonists with neuro-protective properties (Lukiw and Bazan, 2010). Dietary and epidemiological studies have concentrated primarily on effects of dietary HUFA precursors, but have been complemented by pharmacological studies characterizing metabolically active mediators (Cui *et al.*, 2011). Both approaches are important in analysing the actions of rapidly released and metabolized mediators, and cell biology has bridged the gap by analysing metabolism at cellular and system levels, for example, direct effects at the level of lipogenic and peroxiso-

Table 5

Pro- and anti-apoptotic activities of prostaglandin receptor (PGR), and PGR agonists and antagonists, indicating proposed mediators, and selective actions in tumour, normal tissue and tissue/cells affected by degenerative disorders

PGR	Protective	Analogue	Pathology target	Species	Reference
EP1	–	SC-51089	Haemorrhage	Neurones	Rabbit Vinukonda <i>et al.</i> , 2010
EP1	+	17-phenyl trinor PGE ₂	Liver carcinoma	Liver cells	Human Bai <i>et al.</i> , 2010
EP1	–	ONO 8711/ SC 51089	Excitotoxicity	Microglia	Mouse Carlson <i>et al.</i> , 2009
EP1	+	ONO-8713	Medulloblastoma (mb)	Mb cells	Human Baryawno <i>et al.</i> , 2008
EP1	+	EP1 expression	Colon cancer	Colorectal cells	Human O'Callaghan <i>et al.</i> , 2008
EP1	+	17-phenyl trinor PGE ₂	Parkinson's	Neurones	Rat Carrasco <i>et al.</i> , 2007
EP2	+	Soluble EP2 receptor	Bone cancer	Osteoblasts	Human Takahashi <i>et al.</i> , 2008
EP2	+	Butaprost	Medulloblastoma (mb)	Mb cells	Human Baryawno <i>et al.</i> , 2008
EP2	+	Butaprost	Parkinson's	Neurones	Rat Carrasco <i>et al.</i> , 2008
EP3	+	ONO-AE3-240	Oral carcinoma	Squamous cells	Human Hoshikawa <i>et al.</i> , 2009
EP3	+	ONO-AE3-240	Medulloblastoma (mb)	Mb cells	Human Baryawno <i>et al.</i> , 2008
EP4	+	EP4A	T-cell leukaemia	T-cells	Human George <i>et al.</i> , 2007
EP4	+	AH23848B	Adenocarcinoma (ad)	Ad cells	Human Piazuelo <i>et al.</i> , 2006
DP1	+	BW245C/ BWA868C	Cerebral ischaemia	Neurones	Rat Thura <i>et al.</i> , 2009
DP1	+	BWA868C	Colorectal cancer	Crypt cells	Rat Zamuner <i>et al.</i> , 2005
DP1/2	–	PGD ₂	Glioblastoma	A172 cells	Human Payne <i>et al.</i> , 2008
DP2	+	CAY10471	T-cell apoptosis	TH2 cells	Human Xue <i>et al.</i> , 2009
FP	+	PGF _{2α}	Endometrial cancer	Epithelial cells	Human Keightley <i>et al.</i> , 2010
FP	+	Fluprostenol	Skin cancer	JB6 epidermal	Mouse Weber <i>et al.</i> , 2002

mal gene expression (Clarke *et al.*, 1997; see also 'Developing strategies: Hydroperoxy fatty acid signalling and endocannabinoid' sections of this review).

The mechanisms of n-3 HUFA action at cellular level are complex and incompletely understood. Part of their signalling involves substrate specificity for COX and PG synthase, but metabolites of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the resolvins and (neuro) protectins, may also play a part, as they have anti-inflammatory and immunoregulatory actions (Lukiw and Bazan, 2010; Serhan and Haeggstrom, 2010). Compounds derived from EPA (Figure 2) are designated E resolvins, while those formed from DHA are denoted D resolvins or protectins (neuroprotectins). The identification of protectins, which are formed in the presence of aspirin, and are associated with COX acetylation and active site modification, has increased the understanding of drug interactions with biological systems, and biomodulation of metabolism. There is evidence of increased protectin synthesis in pathological processes, for example, neuroprotectin D1 is released in response to ischaemia-reperfusion, oxidative stress or physiological stimulation by neurotrophins. Certain activities of resolvin/protectins are associated with resolution of inflammation, while others appear independent of classical inflammatory cells and pathways (Serhan *et al.*, 2009; Serhan and Haeggstrom, 2010). Like the n-6 PUFA, n-3 HUFA precursors and their lipoxygenase metabolites often have opposing, primarily pro-apoptotic and cell death stimulating activities, while their major COX metabolites are predominantly anti-apoptotic (see topical issues in

eicosanoids section in this review). However, other targets for n-3 HUFA have recently been identified (see 'Developing strategies: PPAR and Endocannabinoid' sections of this review. The n-3 derived endocannabinoids represent a new area of development, which may be especially relevant to tissues and cells in which n-3 HUFA supply is significant).

The role of lipidomics

The cell biology of HUFA signalling has been advanced by improved analytical techniques. Subcellular HUFA release may be analysed using microdissection and mass spectroscopy. Together with other imaging techniques, this provides information on mediator localization and release, spatiotemporal aspects of, for example, mitochondrial signalling and the intrinsic pathway of cell death, and lysosomal activation (e.g. chloroquine, in addition to its recently postulated lysosomal stabilization properties, is also a potent inhibitor of phospholipase A₂).

Prostaglandins and the control of cell death signalling

Lipid metabolites of AA and DHA, the eicosanoids and docosanoids, have been productive targets of pharmacological research. Selective agonists and antagonists with efficacy in cardiovascular disease and anti-inflammatory actions have been developed, and other actions affecting cell death signal-

ling have been identified. The role of eicosanoids in cell death signalling will be discussed in this review. Additionally, lipoperoxidation, PPAR and cannabinoid signalling will be covered, as evidence of their therapeutic potential has emerged.

Prostaglandin signalling may be intracellular or transcellular. Thus, in pathological processes, altered PG metabolism may selectively target the micro-environment, for example, cell and tissue selective HUFA metabolism to PGF_{2α} in endometrial carcinoma, where PGF_{2α} is involved in endothelial cell invasion (Keightley *et al.*, 2010), or loss of prostaglandin D (PGD) synthase in the transition of a low-grade astrocytoma to anaplastic astrocytoma (Payne *et al.*, 2008).

Certain common PGs, present in high concentrations in mammalian tissues and cells (Table 2), have cytoprotective activity, for example, PGE₂ and PGD₂ attenuate neuronal cell death in response to neurotoxic stimuli (Sawyer *et al.*, 2002; Lee *et al.*, 2004; Liang *et al.*, 2005a,b). 15d-PGJ₂ may also be neuroprotective (Lin *et al.*, 2006), and PGE₂ prevented death of neurones in response to TNF-α (Lee *et al.*, 2004). There is current interest in roles of these PGs in angiogenesis and neovascularization (Tables 3 and 5).

Therapeutic aspects of prostaglandin metabolism

Aspirin is the most consumed pharmaceutical agent worldwide and aspects of its activity are still emerging (Warner and Mitchell, 2004). Recently, low-dose aspirin has shown efficacy in cancer trials (Elwood *et al.*, 2009; Rothwell *et al.*, 2011). In an epigenetic analysis of 25 000 patients, analysing death rates and prophylactic treatment with 75 mg·d⁻¹ aspirin, reduced incidence of cancer in gastrointestinal and solid tumours was detected, although the trials were originally set up to study mainly cardiovascular, rather than oncological outcomes (Cuzick *et al.*, 2009). This supports studies suggesting that eicosanoids enhance the ability of cancer cells to resist cell death (Table 5). There is evidence that increased tumour cell proliferation and migration may be associated with prostaglandin E (PGE) synthesis (Rush *et al.*, 2007; Baryawno *et al.*, 2008; Rizzo and Leaver, 2010) and this has implications for angiogenesis (Rizzo, 2011, Table 3). Recent structure/activity analysis of proliferative activity of PGE₂ implicated specific regions of PGE₂, including C5, cyclopentane ring, 9-ketone, C13-14 double bond and 15-hydroxy group (Payner *et al.*, 2006). The signalling pathways affecting key survival decisions affected by nonsteroidal anti-inflammatory drug (NSAID) remain unclear, although the Bcl-2 pathway appears important. Signalling elements have been identified, showing that NSAIDs promoted apoptosis in human HT-1080 fibrosarcoma cell lines by up-regulating p53, p21 and Bax expression, and down-regulating Bcl-2 (Mahdi *et al.*, 2006). Some of these changes have been also observed in glioma cells treated with PUFA (Benadiba *et al.*, 2010; Colquhoun, 2010). It is therefore possible that COX inhibition diverted PUFA into cytotoxic metabolites in fibrosarcoma cells and that this is an effective cytotoxic pathway in transformed cells.

Another topical issue in eicosanoid pharmacology is the relative importance of COX subtypes and the actions of specific COX antagonists. Recent advances in genetic analysis of COX subtypes have led to development of agents targeted against COX-1 and -2 isoforms, which also have activity in

cell death signalling. An aim of NSAID development was inhibition of inducible COX-2 at sites of inflammation, avoiding side effects (e.g. gastric damage) due to inhibition of constitutive COX-1. Although COX-2 selectivity was associated with reduced gastrointestinal damage, COX-2 antagonists also revealed roles for constitutive COX-2 within tissues such as brain, kidney, pancreas, intestine and blood vessels. This has given a better understanding of COX-1 and COX-2 activity in functions as disparate as pain perception and cancer progression (Greenhough *et al.*, 2009). However, clinical use of COX-2 selective compounds has also indicated potential cardiovascular side effects such as myocardial infarction, stroke and elevated blood pressure (Warner and Mitchell, 2004). Also, tumour cells frequently over-express the inducible COX-2 isoform (Rizzo, 2011) and the anti-neoplastic activity of celecoxib was initially assumed to result from selective inhibition of COX-2 and PG synthesis. However, recently celecoxib was also found to inhibit apoptosis in a COX-2-independent manner, which may involve cell death signals and the intrinsic pathway of cell death. Rudner *et al.* (2010) reported that celecoxib induced apoptosis in Jurkat cells via Mcl-1/Noxa, and this effect was inhibited by over-expression of anti-apoptotic Bcl-xL.

Pathology of prostaglandin activity

Prostanoids have been associated with a variety of pathological responses and may act as a primary cellular defence mechanism (Tables 4 and 5). This may be partly due to activation of inflammatory pathways (Portanova *et al.*, 1996), although non-inflammatory actions involving cell death signalling have been observed. During inflammation, PGs may be directly cytoprotective and also act as negative feedback regulators, suppressing cytokine production via JAK/STAT signalling (Tamiya *et al.*, 2011). Gastric mucosa is one of the best characterized tissues with respect to the cytoprotective properties of PGs (Sibilia *et al.*, 2009). However, PGs also suppress cell necrosis in many other tissues in response to chemical and immune-induced cell death, for example, in liver, PGE₂ analogues suppressed cell death in response to galactosamine or complement (Mizoguchi *et al.*, 1987; Kurebayashi and Honda, 1991). More recently, neuroprotective activity of PGs was identified in conditions similar to those following stroke, that is ischaemia reperfusion-induced cell death (Govoni *et al.*, 2001; McCullough *et al.*, 2004), and in systemic inflammatory responses, elevation of PGE₂ in CSF was detected (Davidson *et al.*, 2001). These cytoprotective actions appeared to be mediated, at least in part, via EP2 receptor (EP2R) and intracellular cAMP (McCullough *et al.*, 2004).

Recent advances in cyclooxygenase pharmacology: receptors and signal systems that confer protection by preventing cell death

Pathological PUFA release may exert pro-apoptotic activity via various stress signalling pathways (Figure 1). However, HUFA metabolism via COX is predominantly anti-apoptotic, effectively down-regulating the initial cell stress response.

Figure 3

Prostaglandin structure and signalling via prostaglandin receptors. PGE₂, PGF_{2α} and PGD₂ bind to individual specific membrane-bound cellular receptors, EP1–4, FP and DP1–2, each with specific agonists and antagonists (Table 5). Prostaglandin receptors (PGRs) are coupled via G proteins Gi, Gs and Gq to two principal pathways, linked to either inner plasma membrane-bound adenylate cyclase (AC) or to PLC enzymes. PLC hydrolyses phosphatidylinositol 2 phosphate (PIP₂) to diacylglycerol (DAG) and inositol 3 phosphate (IP₃), while AC cyclizes adenosine triphosphate (ATP) to cAMP. Both pathways are predominantly anti-apoptotic in most cell types. The PGRs and their signalling pathways represent potential molecular sites of selective pharmacological intervention. PGR agonist and antagonist studies also indicate activities independent of these receptors, possibly via additional receptors that have not been fully characterized (e.g. endocannabinoid metabolite receptors), or receptor-independent signalling.

These cytoprotective actions may be partly mediated via cAMP or PLC (Figure 3), although evidence is emerging of actions involving other lipid receptors such as PPAR and endocannabinoid receptors, and cell death signalling pathways involving NF-κB and Bcl. EP2 or DP1 receptors are linked to Gs/adenylate cyclase, and activate cAMP-dependent pathways, such as PKA (Figure 3). The activities of therapeutic agents affecting multiple signalling pathways require careful analysis and systems have been developed for analysing G protein-coupled receptors which initiate downstream signalling (Urban *et al.*, 2007).

Cytoprotective activities of PGE receptors

Many studies have attempted to identify PG receptors involved in preventing cell death, using selective agonists and antagonists (Table 5). These studies have yielded ambiguous interpretations, partly because of overlapping activities with other PG receptors, and also because additional, atypical EP receptors and alternative signalling pathways may exist (Table 5, Bidwell *et al.*, 2010). There are at least four subtypes of PGE₂R, EP1, EP2, EP3 and EP4, linked to different signal systems, with a complex distribution, even within the same cell types (Figure 3, Tables 4 and 5, Andrade da Costa *et al.*, 2009). McCullough *et al.* (2004) used pharmacological and genetic approaches to identify the role of the EP2R. Following focal ischaemia, there was greater infarct volume, with no effect on cerebral blood flow, in EP2R knockout animals. EP2R involvement was supported by neuroprotective actions of the EP2R agonist butaprost (Andrade da Costa *et al.*, 2009; Osborne *et al.*, 2009). Similar cytoprotective effects of PGE₂ were observed in neurodegenerative disease: in the extrinsic pathway involving TNF (Figure 1), Lee *et al.* (2004) showed cytotoxicity to cultured neurones which was ablated by PGE₂. Also, in a cell model of Alzheimer's disease, butaprost (but not EP1/EP3 agonist sulprostone) prevented neurotoxicity in a cAMP-dependent manner following exposure to beta-amyloid protein (Echeverria *et al.*, 2005). Furthermore, in Alzheimer's disease, there was increased PGE₂ in CSF of patients who survived longer (Combrinck *et al.*, 2006) indicating a protective role for PGE₂. This has implications for the design of EP2R selective agonists with neuroprotective activity in neurodegenerative disease and stroke. However, as EP2R is involved in many other functions (Sugimoto and Narumiya, 2007), it may be too general a target.

Cytoprotective activities of PGD and 15-deoxy-PGJ

Recently, PGD₂ has attracted attention as a cytoprotective molecule with fewer potential side effects than PGE₂ (Table 3). PGD₂ is abundant in brain (Hertting and Seregi, 1989), and its receptors may be an appropriate CNS target. Indeed, PGD₂ protected cultured neurones from glutamate-induced toxicity, an action dependent on cAMP (Liang *et al.*, 2005b). Two PGD₂ receptors, DP1 and DP2, have been identified, and the DP1 agonist BW245C mimicked the cytoprotective effects of PGD₂. Similarly, in reperfusion-ischaemia, DP1 receptor knockout animals showed larger necrotic lesions following cerebral artery occlusion, without changes in cerebral blood flow (Saleem *et al.*, 2007; Ahmad *et al.*, 2010). These studies demonstrated protective actions of PGD₂ via DP1 receptors. Thus, DP1R may present another target for therapeutic suppression of neuronal cell death.

A complication in understanding PGD₂ action arises from metabolism of PGD₂ to 15-deoxy-PGJ₂ (15d-PGJ₂), which also has cytoprotective activity (Lin *et al.*, 2006; Ou *et al.*, 2006). 15d-PGJ₂ reduced infarct volume following cerebral ischaemia in mice, coincident with up-regulation of transcription factor PPAR-γ and enhanced nuclear binding of PPAR-γ (Table 4). This suggested that PPARγ mediated some of the cytoprotective actions of 15d-PGJ₂. However, 15d-PGJ₂ may also act

independently of PPAR- γ via cell death signalling pathways. Pereira *et al.* (2006) showed PPAR- γ activation reduced necrosis following cerebral artery occlusion independently of 15d-PGJ₂. Also, 15d-PGJ₂ associated neuroprotection through PPAR- γ -independent mechanisms was reported (Saleem *et al.*, 2007; Thura *et al.*, 2009), and PPAR- γ -independent actions of 15d-PGJ₂ are supported by (i) evidence of 15d-PGJ₂ activity in PPAR- γ knockout cells (Chawla *et al.*, 2001); and (ii) concentrations of 15d-PGJ₂ required to exert an action several orders of magnitude lower than those activating PPAR- γ in the same tissues (Bell-Parikh *et al.*, 2003; Ide *et al.*, 2003). An additional site of action of 15d-PGJ₂ in cell death signalling is nuclear factor NF- κ B signalling (Figure 1). 15d-PGJ₂ reacts with nucleophiles such as free sulfhydryls of glutathione and cysteine residues in cellular proteins, and inhibited activation of NF- κ B via inhibition of phosphorylation and degradation of I κ B α (Ackerman *et al.*, 2005). Indeed, it has also been shown that 15d-PGJ₂ can covalently bind to the cysteine residues of PPAR- γ (Soares *et al.*, 2005).

A gastrointestinal effect of 15d-PGJ₂ has been identified, also involving NF- κ B and Bcl-2 signalling. *Helicobacter pylori* infection, associated with peptic ulcer, gastric atrophy and gastric adenocarcinoma, appears linked to *H. pylori*-induced apoptosis in gastric epithelial cells. Exposure of gastric epithelial cells to *H. pylori* activated transcription factor NF- κ B, which promoted increased pro-apoptotic gene expression (Chu *et al.*, 2003). Recently, Cha *et al.* (2009) demonstrated that 15d-PGJ₂ inhibited apoptosis in *H. pylori*-infected gastric epithelial cells by inhibiting NF- κ B activation, resulting in down-regulation of apoptotic Bax, and up-regulation of anti-apoptotic Bcl-2 gene expression.

Topical issues in eicosanoid pharmacology

Although aspirin and NSAIDs are widely prescribed, their molecular and cellular sites of action are incompletely understood. Recent studies have implicated novel mediators such as the resolvins, PGD₂ and direct actions of HUFA on cell death signalling pathways. The beneficial actions of NSAIDs have been linked to their ability to inhibit COX, and COX-2 selective inhibitor SC58236 exhibited neuroprotective activity in cerebral ischaemia, with marked reduction in lesions (Govoni *et al.*, 2001). This study also showed that ischaemia was accompanied by increased PGD₂, and that COX-2 inhibitor reduced lesions and PGD₂ levels. This is an example of paradoxes reported in the actions of COX inhibitors, that is COX inhibitors being cytoprotective, while the products they inhibit (PGs) may also be cytoprotective! An explanation may lie in COX inhibitor cell death signalling independently of PGE₂ or PGD₂, for example, Vartiainen *et al.* (2001) demonstrated that NS398 (COX-2 selective) and piroxicam (non-selective COX inhibitor) protected neurones following ischaemia-reperfusion-induced necrosis, without up-regulating COX-1 or COX-2, and with little PGE₂ being produced. However, other cytoprotective signalling systems, such as ERK, were activated by COX inhibitors, and it is possible that COX inhibition allowed precursor HUFAs to accumulate. AA has apoptotic activity in many cell types, including leukaemic and vascular cells (Rizzo *et al.*, 1999; 2002; Kalyankrishna *et al.*, 2002; Rizzo and Leaver, 2010). Such PUFA release and signalling would

be transient, as millimolar concentrations of fatty acids are unlikely to accumulate for extended periods, due to rapid re-esterification. The activity and extent of such transient localized signals need further investigation.

Developing strategies: agonist and antagonist design based on substrate specificity and host metabolism: neuroprotectin D1, hydroperoxy fatty acid signalling, endocannabinoids

Analysis of cell death signalling by membrane and lipid mediators has identified potential sites of drug development, ranging from COX metabolism to agonists and antagonists of lysosomal and ceramide signalling pathways. Strategies already discussed include (i) membrane modification via diet, nutraceuticals, specific uptake pathways, often involving n-3/n-6 PUFA modification (Bhathena, 2006; Farooqui and Horrocks, 2006); (ii) the specificity and selectivity of phospholipase A₂, studies extended by recent identification of molecular subtypes and systems which control of their activity (Akiba *et al.*, 2000; Denizot *et al.*, 2009; Sun *et al.*, 2010); (iii) the generation of ROS, including those derived from lipid peroxides, superoxide, nitric oxide (NO being particularly relevant to vascular disease and pathology of endothelial cells), Bcl-2 family proteins acting at the level of mitochondrial permeability, antioxidant functions and Nicotinamide adenine dinucleotide phosphate oxidase (Colquhoun, 2010); (iv) sphingolipid and ceramide pathways (Orgertman and Hannun, 2004; Harr and Distelhorst, 2010); (v) eicosanoids and docosanoids and their receptors (Tables 4 and 5); and (vi) lipoxygenase and platelet activating factor (Esquenazi and Bazan, 2010; Serhan and Haeggstrom, 2010). Additionally, two recently developed areas for therapeutic intervention include the following lipid mediators.

Hydroperoxy-fatty acid signalling

The PPAR nuclear receptors are transcription factors that regulate gene transcription in response to lipid ligands and are involved in cell death signalling (Table 4, Hardwick and Chiang, 2009). The PPAR includes receptors for a wide range of lipids, including steroid and thyroid hormones, vitamin D, retinoic acid, HUFA, HUFA metabolites, and fibrates and thiazolidinedione (TZD) hypolipidemic and antidiabetic agents. PPAR exerts pro- and anti-apoptotic activities in different cells and pathologies. PPAR- γ , the most studied member of the PPAR family, is involved in adipocyte development and is the molecular target for TZD antidiabetic agents. Although PPAR- γ ligands have been useful in treatment of metabolic syndrome, their use is limited by side effects, including oedema, increased plasma volume, adiposity and adverse cardiovascular effects (Ajjan and Grant, 2008). Further analysis of PPAR- γ effects on the kidney and vasculature may help overcome these limitations. PPARs are of pharmacological interest, as they appear to have selective action on transformed cells and cells affected by degenerative disorders (Hardwick and Chiang, 2009). The fatty acid specificity of

PPAR is wide in comparison to cyclooxygenase and lipoxygenase, and PPAR- γ has also been reported to respond to cannabinoids (Giuliano *et al.*, 2009).

Endocannabinoids and their receptors

A novel group of HUFAs containing compounds with therapeutic potential are the naturally occurring cannabinoids, the endocannabinoids, including anandamide (arachidonyl ethanolamide), 2-arachidonoyl glycerol (Pertwee, 2006), O-arachidonyl ethanolamine, 2-arachidonyl glyceryl ether and N-arachidonyl dopamine (Ashton *et al.*, 2008). The reason for the arachidonyl component is unclear, but may be related to the biological activity of this moiety. In addition to the n-6 series of endocannabinoids, n-3 series, specifically docosanoid ethanolamide has also been identified. Bisogno *et al.* (1999) demonstrated the presence of docosahexaenoyl ethanolamide and 2-docosahexaenoyl glycerol in the retina which accumulates DHA. Two receptors associated with endocannabinoid signalling, cannabinoid receptors 1 (CB1) and 2 (CB2), have been identified. Additionally, there is evidence that endocannabinoid metabolites may be effective ligands of PGE receptors (Ross *et al.*, 2002) and of endocannabinoid metabolism via cyclooxygenase and lipoxygenase pathways (Figure 2), and action on vanilloid and capsaicin receptors (Craib *et al.*, 2001; Appendino *et al.*, 2009). CB1 and CB2 are active in cell death signalling pathways. CB1 and CB2 are transmembrane GPCRs which inhibit adenylyl cyclase and activate MAP kinase (see Figure 1). CB1 receptors are present in highest concentration in brain, but are also found in gastrointestinal tract, liver and adipose tissue. CB1 receptors inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels (Pertwee, 2006; 2009; 2010). CB1 receptors are highly expressed in hypothalamic areas involved in food intake. Also, in peripheral tissues, antagonism of CB1 receptors increases insulin sensitivity and oxidation of fatty acids in muscles and liver. CB2 receptors are predominantly located in immune and haematopoietic systems. The discovery of the endogenous cannabinoids led to development of CB1 receptor antagonists in 1994. However, early CB1 antagonists, developed for treatment of obesity, had serious psychiatric side effects, and CB1 antagonists that target peripheral CB1 receptors by restricting their ability to cross the blood brain barrier are currently under development. Possibly of even greater potential are cannabinoid receptor agonists that target the brain, for instance, pain receptor antagonists currently used in chemotherapy-induced nausea and vomiting, relief of neuropathic pain in multiple sclerosis, and agents affecting CB2 receptors in the immune and haematopoietic systems may also be useful (Pertwee, 2009; Bolognini *et al.*, 2010). Recently, it has been shown that n-3 PUFA ethanolamides such as DHA-ethanolamide and EPA-ethanolamide can be antiproliferative towards prostate cancer cells and that part of these actions is mediated via cannabinoid receptors (Brown *et al.*, 2010). It has also been definitively shown that cancer cells possess the capacity to produce DHA-ethanolamide and EPA-ethanolamide (Brown *et al.*, 2011). In developing these agents, better understanding of endocannabinoid pathways, signalling systems and micro-environmental signals modulating their activity is essential,

for example, neuroprotective, anti-apoptotic actions of the phytocannabinoid cannabidiol (Ryan *et al.*, 2009).

Future directions in cell death signalling: membranes, mediators and micro-environments

Strategies in drug design should be informed by signalling pathways at the cellular level. These approaches are being used to investigate the complex biology of cell death. However, genetic and proteomic approaches have diverted attention from the role of membranes in compartmentalization and signalling via membrane metabolism and lipid mediators, especially those associated with HUFA (Figures 1 and 2).

The HUFA is essential for cell function. These epigenetic elements are crucial at cellular level, initiating and integrating key events in cell signalling at the plasma membrane, intracellular organelles, responding to stress signals, and controlling transcription and regulatory factors. HUFA-associated membrane responses and mediator actions are involved in complex pathological processes, and key signalling events associated with disorders of cell death (Table 3). These events are integrated at the level of signal modulation, involving the micro-environment and systems biology (Table 1). Agents affecting HUFA metabolism include the NSAIDs, a pharmacognosy that extends over a century, but which is still yielding insights into the treatment of complex multifactorial diseases (Table 5).

The identity and activity of key mediators is a crucial issue, and novel intermediates associated with prostanoid, cannabinoid, resolvin and endoperoxide pathways are providing new therapeutic opportunities. Topical issues in cell death signalling include how and why membrane metabolism signalling occurs, its role in intracellular and transcellular communication, and interactions with micro-environmental and epigenetic factors involved in pathogenic changes (Table 1). New developments have focused on key initiating events in cell death signalling, interactions at molecular, cellular and system levels, using bioengineering and cell biology.

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Conflicts of interest

None.

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