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Fernanda L.B. Mügge, Aristóbolo M. Silva*

Endoplasmic reticulum stress response in the roadway for the effects of non-steroidal anti-inflammatory drugs

Abstract: Over the past decade, a handful of evidence has been provided that nonsteroidal anti-inflammatory drugs (NSAIDs) display effects on the homeostasis of the endoplasmic reticulum (ER). Their uptake into cells will eventually lead to activation or inhibition of key molecules that mediate ER stress responses, raising not only a growing interest for a pharmacological target in ER stress responses but also important questions how the ER-stress mediated effects induced by NSAIDs could be therapeutically advantageous or not. We review here the toxicity effects and therapeutic applications of NSAIDs involving the three majors ER stress arms namely PERK, IRE1, and ATF6. First, we provide brief introduction on the well-established and characterized downstream events mediated by these ER stress players, followed by presentation of the NSAIDs compounds and mode of action, and finally their effects on ER stress response. NSAIDs present promising drug agents targeting the components of ER stress in different aspects of cancer and other diseases, but a better comprehension of the mechanisms underlying their benefits and harms will certainly pave the road for several diseases' therapy.

Keywords: NSAIDs, PERK, IRE1, ATF6, CHOP, GRP78, XBP1, cancer cells

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***Corresponding authors: Aristóbolo M. Silva:** Laboratory of Inflammatory Genes, Institute of Biological Sciences, Federal University of Minas Gerais – Av. Antonio Carlos, 6627 – UFMG, ICB, Rm J3-243. CEP: 31270-901 Belo Horizonte, MG, Brazil. Phone no.+55 31 3409 2802, Fax no.+55 31 3409 2771. E-mail:aristobolo@icb.ufmg.br

Fernanda L.B. Mügge: Laboratory of Inflammatory Genes, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

1 Introduction

1.1 Players in the ER stress response

Secretory and membrane protein synthesis and ultimate folding to their secondary and tertiary structures are the major functions of the endoplasmic reticulum (ER) in eukaryotic cells. These are critical and essential functions for cell survival and their proper execution is important for the maintenance of homeostasis. Normally, chaperones located in the ER and components from the Endoplasmic Reticulum Associated Degradation (ERAD) are able to deal with unfolded or misfolded proteins, by assisting in the correct folding or by leading to protein degradation, respectively. However, certain stressful stimuli or environmental conditions, like viral infections, lowering in glucose levels, hypoxia, or loss of calcium regulation can result in an increase in unfolded proteins in the ER, the event known as ER stress.

In such perturbation of the ER homeostasis, the Unfolded Protein Response (UPR) is activated as a brisk attempt to restore the ER to its normal function. In this sense, three distinct proteins located in the ER membrane act as the key molecules of UPR signaling, from which downstream molecular events will take place. Activating transcription factor 6 (ATF6), inositol requiring enzyme 1 (IRE1), and protein kinase RNA-like ER kinase (PERK) remain quite inactive under physiological ER functionality, a state that is conferred by the luminal domain binding of these molecules to a master chaperone, GRP78/BiP, abundantly expressed in cells. BiP transiently interacts with so many molecules, especially to newly synthesized proteins as they are translocated into the ER. Certainly, such a formidable, though seemingly promiscuous affinity by other proteins, is fundamental to maintain the ER client proteins in a state competent for subsequent folding and oligomerization. Under stress conditions that result in the accumulation of unfolded polypeptides in the ER, BiP dissociates from PERK, IRE1,

and ATF6 [1,2]. Oligomerization takes place in the case of PERK and IRE1, whereas ATF6 – that is found in monomer, dimer or in an oligomerized state even in unstressed ER [3] – is proteolytically cleaved by the sequential action of proteases.

Although dissociation of BiP from the luminal domains of ER stress sensors seems to be a common stereotype to sense perturbation in the ER, recent studies have provided evidence for a complementary process. This alternate molecular mechanism for sensing ER stress indicates that unfolded proteins may bind directly to luminal domain of Ire1 and favor its dimerization and downstream ER stress signaling [4].

In addition, recent evidence has shown that perturbation of cellular lipid composition is also a trigger for UPR, as PERK and IRE1 have the ability to sense perturbation of membrane aberrancy [5]. The activation mechanism of these ER sensors is independent of their luminal domains, but it is promoted by enhanced dimerization via their transmembrane domain. Such studies uncovered an important mechanistic luminal-independent component that resolved questions regarding the enhanced UPR signaling observed in cholesterol-loaded macrophages and insulin-producing beta cells [6,7].

IRE1 is present in all eukaryotes, being the most evolutionary ancient and conserved of the three arms. Mechanistically, after oligomerization and autophosphorylation IRE1 is activated, which is characteristically evidenced by its kinase and endonuclease (RNase) activities. The endonuclease activity promotes the post-transcriptional processing of the mRNA of X-box binding protein 1 (XBP1), where a cleavage of a residual intron of 26 nucleotides takes place, which will be further degraded. The remaining 5'-PO_4^- and 3'-OH free extremities of the mRNA are reconnected and generate a frameshift of the coding sequence in the mRNA by continuing the protein-coding region into the former 3' untranslated region. Upon translation, a new active and more stable transcription factor, spliced XBP1 (sXBP1), is generated [8]. Once bound to elements in the promoter region of genes that are responsive to ER stress, the transcription is then turned on.

The second arm is mediated by ATF6, a 670 amino acids that gives rise to a 90 kDa protein. Two isoforms, ATF6alpha and ATF6beta are constitutively expressed in various mammalian cell types. After dissociating from BiP, ATF6 is translocated to the Golgi apparatus and is then cleaved by two proteases (S1P and S2P), generating an amino terminal fragment (p50ATF6) that functions as a transcription factor [9-11]. p50ATF6 is translocated into the cell nucleus and activates the transcription of target genes through binding to specific sites, such as ER stress responsive *cis*-acting elements ERSE (CCAAT-N9-CCACG), ERSE II (ATTGG-N-CCACG), and UPRE [GATGACGTG(T/G) NNN(A/T)T] [12-15]. Studies with murine embryonic fibroblasts (MEFs) deficient for ATF6alpha or ATF6beta revealed that only ATF6alpha is necessary to the transcriptional activation of ER chaperones and ERAD components [16].

The transmembrane serine/threonine kinase PERK is present in most mammalian cells and has a predominant expression in pancreatic cells [17]. After activation dependent upon oligomerization, PERK phosphorylates the alpha subunit of mRNA translation initiation factor 2 (eIF2alpha) that leads to the inhibition of mRNA translation in cells. The phosphorylation of eIF2alpha coincides with a clear decline in total cellular protein synthesis [18]. The molecular mechanism as how phosphorylated eIF2alpha provokes protein synthesis inhibition is well established as the reduction in the level of eIF2.GTP.$tRNA_i^{Met}$ ternary complexes in cells limits its availability for the initiation of mRNA translation. In stress conditions such as ER stress, this will adaptively favor cell survival by giving the cell the opportunity to deal with the accumulated misfolded proteins without increasing the loading in the ER. The translation initiation rates for many mRNAs are quite reduced by those events, but exquisitely it can also enhance translation of other mRNAs. This is the case of activating transcription factor (ATF4) in the PERK-mediated pathway where ATF4 mRNA is selectively translated to the extent that eIF2alpha undergoes phosphorylation over cell stress [18]. Thus, PERK-eIF2alpha-ATF4 module contributes to the UPR transcriptional activation, where multiple target genes are transcriptionally activated in a PERK-dependent manner [19-21].

As noted, each of the three aforementioned transcription factors, products of each ER stress branch, is transported to the nucleus and promotes the UPR transcriptional activation. Their targets can be many genes encoding chaperones or ERAD components, intended to alleviate the ER stress. On the other hand, ATF4 and ATF6 might also promote transcription of pro-apoptotic factors like C/EBP homologous protein, CHOP (GADD 153), which is directly related to induced cell death.

The switch of the UPR from a pro-survival strategy to a pro-apoptotic response will depend on the ability of the cell to resolve the ER stress. When the increase in chaperones and ERAD components synthesis is capable of reducing the load of unfolded proteins in the ER lumen, the cell survives. When all strategies fail to do so, the synthesis of pro-apoptotic factors is stimulated and apoptosis is eventually initiated.

Apoptosis induced by prolonged ER stress also depends on the mitochondrial pathway, which is regulated by members of the Bcl-2 family. The activation of the pro-apoptotic proteins Bax and Bak is a key event to caspases activation. CHOP is important in the induction of apoptosis because of the negative regulation of anti-apoptotic Bcl-2 proteins [22]. Additional mechanisms, such as calcium release from the ER and other caspase activation pathways have been implicated in the apoptosis after unresolved ER stress.

2 Non-steroidal anti-inflammatory drugs and modulation of ER stress

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed or bought as over-the-counter (OTC) medicines and commonly used to treat fever, pain, and inflammation. Their classical mechanism of action is the reversible inhibition of cyclo-oxygenase (COX) enzymes, blocking prostaglandins (PG) production from arachidonic acid (a membrane phospholipid). Arachidonic acid is converted to PGG2 by COX, which also catalyzes the generation of PGH2 from PGG2. PGH2 can be converted later to other PGs or thromboxane A2 (TXA2) by specific enzymes. As an exception, acetyl salicylic acid is the only NSAID that causes an irreversible inactivation of COX after its acetylation [23].

Two different isoforms of COX can be modulated by NSAIDs. Inhibition of COX-2 is related to the analgesic, antipyretic, and anti-inflammatory actions, since it is induced by the inflammatory process. Because of its constitutive expression, inhibition of COX-1 causes, but not exclusively, the most common NSAIDs adverse effects, mainly related to the gastrointestinal tract. The majority of the available drugs are non-selective COX inhibitors, thereby acting on both enzymes [24].

The different classes of NSAIDs (shown in Table 1) are not chemically related and even inside the same group some molecular modifications generate drugs with different pharmacological profiles. Also, they may differ on toxicity profiles and COX-1/COX-2 selectivity.

The pharmaceutical industry focused for some time on the development of specific COX-2 inhibitors (Coxibs) as a strategy to reduce or avoid the occurrence of adverse effects. Coxibs are a class of selective COX-2 inhibitors displaying lower gastrointestinal toxicity with comparable anti-inflammatory action to other classes. A few years after their release, some coxibs were withdrawn from the market (Rofecoxib and Valdecoxib) because of the induction of higher cardiovascular risks and low safety during prolonged use. Others drugs of this class, however, are still commercialized in many countries, including the US (celecoxib), but are not prescribed as first choice drugs [25].

Acetaminophen, also known as paracetamol, lacks anti-inflammatory actions, and sometimes is not considered to be a NSAID [23]. Its exact mechanism of action is not yet elucidated, but many hypotheses have been made regarding its central or peripheral actions. It has been shown to inhibit a third COX isoform, COX-3 in canine cerebral cortex [26], but a functional COX-3 has not been found in rodents or humans [27]. Based on its similar pharmacological profile to selective COX-2 inhibitors, like lower gastrointestinal toxicity and no platelet inhibition, more recent studies have demonstrated selective actions on COX-2 in clinically relevant models. In one of these studies, the investigators showed an up-regulation of the COX-2 gene and suppression of PGE2 in inflammatory transudate collected from sites of acute inflammation after 1 g acetaminophen intake by patients [28]. In another study, COX-2 was inhibited by 80% in peripheral blood

Table 1: Classes of non-steroidal anti-inflammatory drugs (NSAIDs)

Class	Members
Salicylates	Acetyl salicylic acid
	Sodium salicylate
	Diflunisal
	Mesalazine/Mesalamine
Acetic acid derivatives	Indometacin
	Sulindac
	Diclofenac
	Etodolac
Propionic acid derivatives	Ibuprofen
	Flurbiprofen
	Ketoprofen
Anthranilic acid (Fenamate)	Mefenamic acid
	Niflumic acid
Enolic acids (Oxicams)	Piroxicam
	Meloxicam
Sulfonanilide	Nimesulide
p-Aminofenol derivatives	Paracetamol/Acetaminophen
Diarylheterocycles (Coxibs)	Celecoxib
	Parecoxib
	Rofecoxib
	Valdecoxib

Adapted from [23]

monocytes after 1 g acetaminophen administration to volunteers [29].

In spite of several proposed mechanisms of action, there is accumulated evidence that acetaminophen exerts remarkable effects on ER stress response, and is clearly associated with tissue injury, especially in the liver and kidney, provoked by this compound. Such ER stress-mediated harm effects of acetaminophen will be properly discussed in the section of NSAIDs toxicity.

Since the clinical studies reported by Edmund Stone in 1763 on the effects of willow-bark powder used to treat patients suffering from ague [30] and subsequent numerous studies for purification and synthesis, salicylates have long been used in an impressive effective manner for several disease conditions and disorders. Besides their common use as anti-inflammatory, anti-pyretic and analgesic, they have also been used for the prevention of cardiovascular diseases and cancer [31,32]. The prototype salicylate is acetylsalicylic acid, the popular aspirin. The effects of salicylates on ER stress components have revealed that these compounds affect the activity and expression of several molecules related to ER stress [33,34], and triggering fundamental cellular processes, including transcriptional activation of ER stress responsive genes [35].

Various other studies that have exploited the effects of NSAIDs on ER stress are largely descriptive, but the demonstration of several common targets that have their activity affected by different classes of NSAIDs can pave the road for further mechanistic studies on how ER stress response is affected (Table 2). These compounds and their effects on ER stress will now be discussed.

2.1 NSAIDs toxicity

2.1.1 Gastrointestinal toxicity

2.1.1.1 COX inhibition

As mentioned previously, NSAIDs use is commonly associated with gastric toxicity, partially because of their acidic characteristics and the inhibition of COX-1. This enzyme is responsible for the production of cytoprotective PGs by the cells, which contribute to the maintenance of mucosal integrity through bicarbonate and mucus production stimulation. The decrease of these protective substances leads to higher cell permeability and promotes direct contact of the cells with the acidic gastric secretion, causing injuries [23].

Patients with increased sensitivity to NSAIDs or those who make a long-term use of high doses of NSAIDs usually show more severe lesions that can result in ulcers, bleeding and sometimes perforations, often aggravated by platelet inhibition due to reduced TXA2 production from PGH2 as a result of COX-1 inhibition (reviewed in [36]).

The mechanisms of NSAID-induced enteropathy have some similarities to those related to gastric injuries, and a "multiple hits" effect is currently accepted. The combination of reduced PGE2 levels by COX inhibition and topical effects resulting in mitochondrial dysfunction and stress responses would be the "first hit", while the subsequent inflammatory response would be responsible for the second stage of NSAID-induced small intestine injuries (reviewed in [37]).

As reported by several studies, COX-1 inhibition does not entirely explain the occurrence of NSAID induced gastrointestinal lesions. It has been shown that a selective COX-1 inhibitor did not cause any gastric injury even in high dosage, which only happened when this drug was associated with celecoxib, a COX-2 selective inhibitor [38]. Additionally, COX-1 knockout (KO) mice have no gastric pathology and are also more resistant to indomethacin induced gastric lesions than wild type (WT) mice [39].

2.1.1.2 Cytotoxic mechanisms

In two studies, Tomisato *et al.* [40,41] have provided evidence of the induction of necrosis and apoptosis by NSAIDs in the gastric mucosa. They showed in the first study that exposure of primary cultures of guinea pig gastric mucosal cells to indomethacin can cause either necrosis, when the cells were treated with a high dose (2.5 mM) for a short period of time (1 hour), or apoptosis, when cells were treated with lower doses (between 0.5 and 1 mM) for a longer period of time (16 hours). Necrosis was evidenced by propidium iodide (PI) staining of cell nucleus, while apoptosis was characterized by DNA/nucleus fragmentation and also by examining the activation of caspases 3, 8 and 9. Treatment of cells with caspase inhibitor z-VAD-fmk did not alter cell viability in conditions that led to necrosis, but inhibited DNA fragmentation in lower doses and higher time of exposure to indomethacin. Interestingly, pre-treatment of cells with cycloheximide, a protein synthesis inhibitor, led to increased cell viability in the conditions shown to induce apoptosis. In the second study, the authors suggest that induction of necrosis or apoptosis is a COX independent mechanism, being the result of the direct cytotoxicity of NSAIDs on gastric cells, and that gastric lesions may be the result of both COX inhibiting activities and cytotoxicity of NSAIDs.

Table 2: Summary of NSAIDs actions on ER stress components

NSAID	Cell/Tissue/Organ	Effect on ER stress	References
Indomethacin	Primary gastric mucosal cells	↑CHOP ↑GRP78 ATF6 activation ↑ATF4 mRNA ↑XBP1 mRNA expression and splicing ERSE activation	[42]
	Hepatoma cell line	↑GRP78 mRNA (slight) ATF6 activation XBP1 splicing (partially) eIF2α phosphorylation ↑CHOP	[54]
	Renal carcinoma cells	↑CHOP ↑GRP78 ↑GRP94 ATF6 activation	[74]
Diclofenac	Primary gastric mucosal cells	↑CHOP ↑GRP78	[42,43]
	Rat hepatocytes	↑CHOP	[53]
	Hepatoma cell line	↑GRP78 mRNA (slight) ↓p90 ATF6 ATF6 activation eIF2α phosphorylation ↑CHOP	[54]
Acetaminophen	Liver from CD-1 mice after intraperitoneal injection	↑CHOP ATF6 activation	[56]
	Liver from C57BL/6J mice after oral administration	↑ATF4 ↑ATF3 ↑CHOP ↑XBP1 XBP1 splicing ATF6 activation IRE1α phosphorylation eIF2α phosphorylation	[58]
	Tubular epithelial cells	↑CHOP expression and translocation to the nucleus	[59]
	Organ of Corti cell line	↑eIF2α phosphorylation ↑CHOP expression and nuclear translocation	[60]
Ibuprofen	Primary gastric mucosal cells	↑CHOP	[42]
	Neuroblastoma cell line	↑ATF4 ↑ATF6 ↑XBP1	[73]
Pranoprofen	Primary glial cells	↓GRP78 ↓CHOP eIF2α phosphorylation ↓XBP1 splicing	[90]
Flurbiprofen	Neuroblastoma cell line	↓XBP1 splicing ↓CHOP expression ↓HERP expression	[86]

continued **Table 2:** Summary of NSAIDs actions on ER stress components

NSAID	Cell/Tissue/Organ	Effect on ER stress	References
Celecoxib	Primary gastric mucosal cells	↑CHOP	[42]
	Glioblastoma cell lines	↑CHOP ↑GRP78 eIF2α phosphorylation	[47,64,68]
	Raji Burkitt's lymphoma cells	↑CHOP	[65,68]
	Breast carcinoma cells	↑CHOP	[68]
	Pancreatic carcinoma cells	↑CHOP	[68]
	Multiple myeloma cells	↑CHOP	[68]
	Gastric carcinoma cells	↑GRP78 eIF2α phosphorylation PERK phosphorylation ↑ATF4 ↑ATF6 mRNA ATF6 activation ↑ORP150	[48,50,72]
	Colorectal cancer cells	↑GRP78	[69]
	Urothelial carcinoma cells	↑GRP78	[70]
	Hypopharyngeal squamous cancer cell line	↑CHOP ↑GRP78 ↑XBP1	[67]
Celecoxib + bortezomib	Glioblastoma cell line	↑CHOP ↑GRP78	[78]
Celecoxib + MG132	Urothelial carcinoma cells	↑CHOP ↑GRP78	[70]
	Liver tumor cells	↑CHOP ↑ATF4 ↑XBP1 splicing	[79]
Celecoxib + PDE5 inhibitor	Multiple tumor cells	eIF2α phosphorylation	[81]
2,5-dimethyl-celecoxib (DMC)	Glioblastoma cell lines	↑CHOP eIF2α phosphorylation	[47,64,68]
	Raji Burkitt's lymphoma cells	↑CHOP	[65,68]
	Breast carcinoma cells	↑CHOP	[68]
	Pancreatic carcinoma cells	↑CHOP	[68]
	Multiple myeloma cells	↑CHOP	[68]
DMC + nelfinavir	Breast cancer cells	↑CHOP ↑GRP78	[80]
DMC + nelfinavir + chloroquine or mefloquine		↑↑CHOP ↑↑GRP78	[84]
Parecoxib	Rat brains after ischemic injury	↑GRP78 ↑ORP150 ↓CHOP expression and nuclear translocation	[92]
Sulindac sulfide	Intestinal cancer cells	↑GRP78 ↑CHOP ↑GADD34 ↑ATF4 ↑ATF3	[76]
	Glioma cells	↑GRP78	[77]

continued **Table 2:** Summary of NSAIDs actions on ER stress components

NSAID	Cell/Tissue/Organ	Effect on ER stress	References
Meloxicam	Rat brains after ischemic injury	↓CHOP mRNA ↓GRP78 mRNA and protein ↓GRP94 mRNA	[91]
	Cerebral cortex	↑GRP78	[91]
Oxicams (meloxicam, sudoxicam, piroxicam)	Rat hepatocytes	↑CHOP	[53]
Nimesulide	Rat hepatocytes	↑CHOP	[53]
Fenamic acids (flufenamic, tolfenamic and mefenamic acids)	Rat hepatocytes	↑CHOP	[53]
Diflunisal	Rat hepatocytes	↑CHOP	[53]
Tolfenamic acid	Colorectal cancer cells	↑ATF6 activity ↑CHOP ↑ATF4 ↑XBP1 splicing	[71]
Aspirin	Immortalized mouse embryonic fibroblast	eIF2α phosphorylation PERK phosphorylation no XBP1 splicing	[34]
Sodium salicylate	Immortalized mouse embryonic fibroblast	eIF2α phosphorylation ↑CHOP caspase-12 degradation no XBP1 splicing	[34]
		↑34 ER-stress related genes ↑ATF6 ERSE activation	[35]
	Human adipocytes	↓ATF6 ↓CHOP ↓GRP78	[33]

↑: increased expression; ↓: decreased expression; ↑↑: higher increased expression. ATF, activating transcription factor; CHOP, C/EBP homologous protein; eIF2, eukaryotic translation initiation factor; ERSE, ER stress response element; GRP, glucose regulated protein; IRE1, Inositol-requiring endonuclease; ORP150, 150-KDa oxygen regulated protein; PERK, protein kinase R (PKR)-like endoplasmic reticulum kinase; XBP1, X-box binding protein.

2.1.1.3 ER stress in NSAIDs-induced effects of cytotoxicity

Further investigations revealed that NSAIDs exposure of cells could disturb the homeostasis of the ER by modulating the ER stress response, and that could be an upstream mechanism responsible for apoptosis and gastrointestinal injuries elicited by these drugs. One of the first studies in this front was carried out by Tsutsumi and colleagues [42]. In primary cultures of guinea pig gastric mucosal cells, indomethacin (1 mM) was shown to induce CHOP mRNA and protein levels in greater extent than thapsigargin, a well-known ER stress inducer agent that disrupts calcium regulation in the ER. Diclofenac (1 mM), ibuprofen (2 mM), and celecoxib (80 μM) also displayed effects on those cells such as reduced cell viability, increased DNA fragmentation, and an increase in CHOP protein levels in a COX independent manner. Other stress agents that cause apoptosis, such as ethanol, hydrogen peroxide, and hydrochloric acid lack the ability to induce an increase in CHOP mRNA levels, showing that this event is specific for NSAID-induced apoptosis. In the same study, levels of GRP78 were slightly increased after indomethacin treatment [42].

In a toxicoproteomic approach, an increase in GRP78 levels induced by a NSAID was also reported by Ohyama *et al.* [43] in a study carried out in mice treated with

diclofenac indicating that the ER stress response takes place. Such finding corroborates the notion that NSAID-induced gastrointestinal toxicity is mediated by ER stress components. Back to study of Tsutsumi and colleagues, ATF6 was activated by indomethacin, since p90ATF6 levels decreased in whole cell extracts and p50ATF6 levels increased in nuclear extracts. Both ATF4 and XBP1 mRNA expression, including the spliced sXBP1 and JNK1/2 phosphorylation were induced by indomethacin. Accordingly, transfection of cells with ERSE or CHOP binding-site luciferase reporter plasmids showed transcriptional activation and luciferase activity after indomethacin or thapsigargin treatment, at comparable extensions. These results indicate that ATF6, ATF4 and XBP1 might be responsible for the expression of CHOP induced by indomethacin, and also that downstream events to ASK1, which results in JNK phosphorylation, seem to be activated by indomethacin. Additionally, experiments with a dominant-negative form of CHOP or in macrophages derived from CHOP-deficient mice indicated that apoptosis induced by NSAIDs involves other components than CHOP, as suppression of apoptosis was only partially dependent on this transcription factor [42].

In a proposed mechanism for NSAID induced apoptosis, NSAIDs would promote cell membrane permeabilization, due to their ability to interact with membranes and cause alterations in hydrophobicity, fluidity, biochemical properties, and stability (reviewed in [44]). Higher permeability would stimulate calcium influx, leading to increased calcium levels in the cytoplasm, which then could culminate in ER stress. Indomethacin, diclofenac, etodolac, ibuprofen, flurbiprofen, ketoprofen, celecoxib, mefenamic acid, and nimesulide were shown to increase membrane permeability and intracellular calcium levels and to decrease cell viability in guinea pig gastric mucosal cells. These events were partially inhibited when a chelating agent was added before to celecoxib treatment [45]. As described, NSAID-induced ER stress promotes significant increase in the CHOP mRNA and protein expression, which is an alternate route to apoptosis. A molecular link between CHOP and mitochondrial dysfunction after NSAID exposure was suggested to be the pro-apoptotic modulator PUMA, as its silencing mediated by siRNA led to reduction in the celecoxib-induced activation and translocation of Bax that is a critical protein that regulates mitochondrial outer membrane permeabilization. Silencing of ATF4 but not ATF6 was shown to decrease PUMA mRNA expression when cells were treated with celecoxib. In similar conditions, CHOP mRNA expression was slightly reduced by ATF6 silencing but more extensively reduced when ATF4 was silenced [46]. From described above and additional studies, it is clear that celecoxib preferentially targets PERK-eIF2alpha-ATF4 module [47,48].

2.1.1.4 Exacerbation of NSAIDs-induced gastric effects through chaperones suppression

Helicobacter pylori infection, another major cause of gastric ulcers, can also be a predisposition factor for NSAID-induced gastric injuries. These bacteria can produce irritating substances or down regulate the production of protective factors by gastric cells. Namba *et al.* [49] demonstrated that *H. pylori* exacerbates NSAID-induced gastric lesions by suppression of ER chaperones expression. An increase in the expression of GRP78 and ORP150 (150-KDa oxygen regulated protein) was previously shown to reduce CHOP triggered apoptosis in cells after celecoxib treatment [48,50], indicating a protective role for these chaperones against NSAIDs-induced apoptosis. Co-culture of human gastric carcinoma cells with *H. pylori* decreased GRP78 and ORP150 levels in a time-dependent manner, and strongly reduced p50ATF6 and p90ATF6 expression after 24 or 48 hours, respectively. Levels of ATF4 remained unaltered and XBP1 splicing did not take place. Yet, silencing of ATF6 with siRNA resulted in decreased expression of GRP78 and ORP150, but not CHOP. Expression of ATF6 mRNA was not regulated by *H. pylori*, but a pulse-chase experiment indicated that degradation of ATF6 is accelerated in the presence of *H. pylori*, suggesting a post-translational mechanism for the regulation of ATF6, probably by proteasome and/or lysosome degradation. In mice pre-inoculated with *H. pylori*, the indomethacin-induced gastric lesions were aggravated, accompanied by an increase in TUNEL-positive cells compared to indomethacin treatment alone. Furthermore, ORP150-/+ heterozygous mice showed more apparent gastric mucosal cell death, providing genetic evidence for the protective role of ER chaperones in indomethacin-induced gastric lesions [49]. This suggests that molecules capable of inducing ER chaperones may offer therapeutic advantage against NSAIDs-induced gastric lesions.

2.1.2 NSAIDs-induced ER stress to hepatotoxicity

Another concern for individuals who make use of NSAIDs is the hepatotoxicity effects caused by some of these drugs. Although seeming to be a rare complication, some severe reactions were the cause for the withdrawal of some NSAIDs. Aspirin, celecoxib, diclofenac, ibuprofen,

indomethacin, naproxen, nimesulide, piroxicam, and sulindac have been reported to cause hepatotoxicity with different characteristics, varying from elevation of transaminases, cholestasis and hepatitis, to acute liver failure in some cases (reviewed in [51]). Bromfenac, only four months after its availability to patients, was reported to cause acute liver failure requiring transplantation and even leading to death, which resulted in its withdrawal less than one year after its approval. Lumiracoxib, a selective COX-2 inhibitor chemically related to diclofenac, was never approved by the FDA in the US and was withdrawn from the market because of its association with severe drug induced liver injury [52].

In a toxicity assessment of various NSAIDs, rat hepatocytes were used as one of the models to evaluate the hepatotoxicity of different chemical classes: three fenamic acids (flufenamic, tolfenamic, and mefenamic acid), three oxicams (meloxicam, sudoxicam, piroxicam), two salicylates (aspirin and diflunisal), two acetic acid derivatives (diclofenac and sulindac), and the sulfonanilide, nimesulide. Except for acetyl salicylic acid and sulindac, all of the other aforementioned NSAIDs induced CHOP expression in these cells [53].

In a hepatoma cell line, diclofenac and indomethacin were shown to trigger the activation of the different branches of the UPR. Semi quantitative RT-PCR indicated a slight induction of GRP78 mRNA, after 8 hours of treatment, but less pronounced than that observed for thapsigargin. Full-length p90ATF6 and cleaved p50ATF6 were present in cells treated with indomethacin in a similar profile in response to thapsigargin. Regarding diclofenac, however, the expression of p90ATF6 was decreased and even lower than in control untreated cells, while p50ATF6 was present in cell extracts but in a lower intensity than indomethacin or thapsigargin treatments. Only indomethacin was shown to partially promote XBP1 splicing after 45-60 minutes of treatment, as evidenced by the RT-PCR analysis of sXBP1 amplicons in agarose gels. On the other hand, diclofenac, but not indomethacin or thapsigargin, induced JNK phosphorylation after 16 h, indicating different actions of these drugs on IRE1. Phosphorylation of eIF2alpha was also evidenced in cells treated with indomethacin or diclofenac, likely indicating PERK activation. Moreover, CHOP expression was induced by both drugs, but with higher intensity for indomethacin than for diclofenac or thapsigargin after 8 hours of treatment. Finally, flow cytometry analysis revealed that diclofenac and indomethacin induce cell death as similar as thapsigargin [54].

Acetaminophen, which is widely used to treat fever and pain, is a major cause of acute liver failure. Its exact mechanism of hepatotoxicity is not known, but it clearly involves necrosis and evidence suggest that apoptosis is also involved. Caspase-dependent Bid cleavage and caspase-independent Bax translocation to mitochondria and cytochrome c release were reported after 6 h of acetaminophen administration to BALB/c mice, but caspases 3 and 7 were not activated. Caspase inhibitors, however, protected mice from acetaminophen-induced liver injury, suggesting that apoptosis would be the initial cell death response to acetaminophen treatment, but later it would turn to degeneration and necrosis [55].

The ER is the primary site of acetaminophen metabolism that is dependent on its oxidative biotransformation to NAPQI, which leads to glutathione (GSH) depletion, an event supposed to be related to its toxicity. Perturbations of the redox homeostasis can impair the oxidative folding of proteins in the ER, which may result in ER stress. Nagy *et al.* [56] demonstrated CHOP induction and ATF6 activation after intraperitoneal administration of acetaminophen (450 mg/Kg) to male CD-1 mice. On the other hand, Hur *et al.* [57] did not observe PERK or ATF6 activation in the liver of mice that received intraperitoneal administration of acetaminophen (500 mg/Kg). In this same study, genetic ablation of XBP1 was found to protect mice from acetaminophen-induced liver injury through IRE-1alpha activation via a feedback mechanism not related to UPR activation [57]. Divergent observations by these studies may reflect the use of different mice backgrounds or gene targeting strategies.

Because intraperitoneal injection of acetaminophen does not reproduce the actual route of administration in patients, a third study evaluated the induction of UPR after oral administration (gavage) of acetaminophen 500 mg/Kg to mice. Gene expression analysis revealed induction of ATF4 and its targets, ATF3 and CHOP, and of XBP1 and its targets, ERdj4 and $p58^{ipk}$, in animals treated with acetaminophen. XBP1 mRNA splicing, p90ATF6 cleavage, IRE1alpha, eIF2alpha phosphorylation, and CHOP protein expression were only observed at 12 hours after acetaminophen administration. Additionally, CHOP KO mice were shown to be more resistant to necrosis progression after acetaminophen gavage, having ten-fold lower necrosis areas than wild-type mice after 24 hours of drug administration. CHOP-deficient mice had also improved survival, manifested with 100% survival with a dose of 1 g/Kg acetaminophen, which led to death within 24 hours of all wild-type animals administered with the same dose. Levels of GSH and JNK phosphorylation, which occurs downstream of GSH depletion, were similar in both groups, indicating that the protection observed in CHOP deficient mice was not a result from

the reduced acetaminophen metabolism. Furthermore, it was demonstrated that CHOP deficiency was beneficial for recovery from ER stress, since knock-out animals presented lower levels of ER stress markers than controls, and displayed increased capacity of regeneration at the sites of damage [58].

2.1.3 NSAIDs-induced ER stress to nephrotoxicity

Besides hepatotoxicity, acute acetaminophen overdose can cause nephrotoxicity through molecular mechanisms still to be fully elucidated. In culture mouse tubular epithelial cells, acetaminophen was shown to induce apoptosis, which was dose and time dependent. Despite increase on Fas receptor expression on cell surface, this pathway did not seem to be activated, as blocking of the receptor caused no alterations in the rate of apoptosis induction. Also, there was neither cytochrome c nor Smac/DIABLO release from mitochondria nor loss of mitochondria membrane potential. However, acetaminophen induced the activation of caspase 3, 9 and 12, and CHOP expression and its consequent translocation to the nucleus. The authors suggest that the mechanism of acetaminophen-induced nephrotoxicity is caspase dependent. In addition, caspase 12 that is highly expressed in the kidneys and present in the ER membrane could be a link between ER stress and apoptosis caused by acetaminophen in tubular cells [59].

2.1.4 NSAIDs-induced ER stress to ototoxicity

Authors of a recent study refer to the ototoxicity of acetaminophen and its metabolite N-acetyl-p-benzoquinoneimine (NAPQI) as a relevant side effect of these drugs. They suggest that ototoxicity is masked by the hepatotoxicity because the latter leads to death before any hearing loss can be perceived. Using a cell line derived from the organ of Corti (HEI-OC1), these authors demonstrated that the ototoxic effect involves oxidative and ER stress pathways, through overproduction of ROS, alterations in ER morphology and modulation of the eIF2α pathway. CHOP expression and nuclear translocation were demonstrated through immunofluorescence microscopy. Acetaminophen treatment of cells led to an increase in eIF2α phosphorylation while NAPQI led to decreased phosphorylation. Silencing of ATF4 with shRNA, or through pharmacological inhibition of ATF6 activation in the Golgi (using 4-(2-aminoethyl) benzenesulfonyl fluoride) caused no alterations in cell viability after acetaminophen or NAPQI treatment. XBP1 splicing was not induced by treatment with these drugs [60].

2.2 Therapeutic applications

2.2.1 Cancer treatment

Epidemiological studies have indicated a role for NSAIDs in the prevention of cancer, while selective COX-2 inhibitors have been shown to inhibit tumor growth and metastasis in different animal models of solid tumors. Indeed, COX-2 is overexpressed in some tumors and the final products of the conversion of arachidonic acid to prostaglandins, namely PGE2, PGI2, PGD2, PGF2alpha and TXA2, promote cell proliferation, metastasis and angiogenesis, and inhibit apoptosis. Therefore, inhibition of COX-2 could have beneficial effects in the prevention and treatment of cancer (reviewed in [61,62]).

It is now known that, in a similar way to adverse effects described for NSAIDs, both COX-dependent and COX-independent pathways play roles in the induction of apoptosis in cancer cells. The underlying mechanisms are not completely understood, but increasing evidence suggest modulation of different intracellular components related to cell cycle, apoptosis, inflammation and also ER stress [61,63].

2,5-dimethyl-celecoxib (DMC), a celecoxib analog that possesses no COX inhibitory activity, has been shown to have more potent tumor suppression properties than celecoxib, while unmethylated celecoxib (UMC), which has slightly higher COX-2 inhibitory activity, possesses lower antitumor effect in glioblastoma cell lines [64] and in Raji Burkitt's lymphoma cells [65]. DMC concentrations starting at 50 μM decreased cell viability in a proportion that was only observed at 70 μM celecoxib and that was not achieved by UMC. CHOP expression and caspase activation after celecoxib or DMC correlates with reduced cell survival in both cell types and is not induced by UMC treatment [64,65].

An increase in cellular protein synthesis can be associated with an increase in cell proliferation, thus making translational machinery a potential target for tumor therapy. In this sense, anti-proliferative effects of NSAIDs via ER stress components could have PERK-eIF2alpha module as target. Celecoxib was found to transiently inhibit protein synthesis in glioblastoma cells in a dose-dependent manner. In this study carried out by Pyrko *et al.* (2008), celecoxib at roughly 80 μM concentration inhibited protein synthesis from 2 to 8 hours of treatment, a time when protein synthesis began to recover, being reestablished after 24 hours treatment.

Phosphorylation of eIF2alpha was observed in the same doses and treatment periods demonstrated to induce translation inhibition. In mouse embryonic fibroblasts (MEFs) that express a mutant eIF2alpha, which cannot be phosphorylated (serine 51 is exchanged for alanine), protein synthesis inhibition by celecoxib was reduced in relation to control cells. Interestingly, other NSAIDs (rofecoxib, valdecoxib, indomethacin, and flurbiprofen) in 100 μM doses were unable to inhibit protein synthesis, cell growth survival, or induce eIF2alpha phosphorylation. DMC, on the other hand, induced more pronounced eIF2alpha phosphorylation than celecoxib. These latter results indicate that inhibition of mRNA translation by celecoxib is another COX-independent mechanism of this drug and provide a strong evidence of the involvement of PERK in the celecoxib-induced cell effects [47]. Moreover, increased GRP78 expression was evidenced by immunoprecipitation study from extract of cells that were treated with 75 μM celecoxib for 12 hours, indicating another ER stress related event induced by celecoxib.

Aspirin and sodium salicylate have also been shown to inhibit protein synthesis in a mechanism dependent on PERK activation and eIF2alpha phosphorylation, which was demonstrated using mouse embryonic fibroblasts (MEFs) lacking PERK. In addition, aspirin and sodium salicylate induced significant CHOP protein expression, which was attenuated in PERK-/- cells. These two drugs also induced caspase-12 degradation but had no effect on XBP1 splicing [34]. Hence, it is likely that PERK is a critical ER stress component in cell responses to salicylates.

In PC-3 cells, a prostate cancer cell line, celecoxib induces an increase in calcium intracellular levels in a dose-dependent manner. Although not shown in their paper, the authors mentioned that the same increase in intracellular calcium levels was observed in another prostate cancer cell line (LNCaP), in smooth muscle cells (A7r5), in NIH-3T3 fibroblasts, MCF-7 breast cancer cells, Jurkat T cells, and HepG2 hepatoma cells. According to them, this could be a possible link between celecoxib anti-cancer activities and increased cardiovascular risks [66]. A recent report has also supported the notion of ER stress-mediated antiproliferative effects of celecoxib. In that work, the authors demonstrate that by knocking-down CHOP hinders the inhibition of cell proliferation by celecoxib in a well established human hypopharyngeal squamous cancer cell line [67].

In different cancer cell types, such as glioblastoma, breast carcinoma, pancreatic carcinoma, Burkitt's lymphoma, and multiple myeloma, celecoxib and DMC were shown to induce CHOP protein expression, with a clear effect that was dependent on the cell type. In glioblastoma cells, the induction of CHOP was determined to be specific to these two drugs, since it was not evidenced after valdecoxib or rofecoxib treatments. Also, in glioblastoma cells that had been implanted into nude mice, CHOP expression as well as the number of TUNEL positive cells were increased by celecoxib and DMC, but not by rofecoxib. Since both celecoxib and DMC led to an increase in calcium intracellular levels and inhibition of DMC-induced CHOP expression in the presence of chelating agents, calcium was again suggested to be an important trigger to the induction of ER stress by these two drugs [68]. However, other NSAIDs including valdecoxib, rofecoxib, indomethacin, flurbiprofen, and sulindac did not induce any increase in calcium intracellular levels. Discrepancy effects seen for some NSAIDs [45] may be due to the cell types used [45].

In the previous study by Pyrko *et al.* [68] and in studies by Tsutsumi *et al.* [48], Du *et al.* [69], and Huang *et al.* [70], where glioblastoma, gastric carcinoma, colorectal cancer, and urothelial carcinoma cells were used, respectively, GRP78 was implicated as a protective molecule against celecoxib induced apoptosis. In all three studies celecoxib was shown to increase GRP78 expression, but its knock-down with siRNA led to enhancement of glioblastoma cell killing [68] and increased caspase 3, 7, 8 and 9 cleavage in urothelial carcinoma cells [70] after celecoxib treatment. In the colorectal cancer cells, overexpression of GRP78 significantly reduced the rate of apoptosis induced by celecoxib [69]. Interestingly, tolfenamic acid also exerts remarkable effects in colorectal cancer cell growth both *in vitro* and *in vivo* by repressing cyclin D1 expression in a PERK-eIF2alpha-ATF4-dependent manner. In these cells, tolfenamic acid was also shown to induce higher ATF6 activity, XBP1 splicing and higher expression of CHOP and ATF4 [71]. At least in the gastric carcinoma cells, celecoxib induces eIF2alpha and PERK phosphorylation, which results in ATF4 expression. Because ATF4 silencing using siRNA partially reversed the overexpression of GRP78 induced by celecoxib, this seems to be one of the UPR arms responsible for GRP78 up regulation after celecoxib treatment [48].

Together with ATF4, ATF6 was also linked to the celecoxib-induced up regulation of another ER chaperone, ORP150, in gastric carcinoma cells. Silencing of both transcription factors using siRNA inhibited ORP150 overexpression by celecoxib [72]. ATF6 expression and activation was shown to be induced with 80 μM or 100 μM celecoxib in these cells, as demonstrated by the increase in ATF6 mRNA relative expression [50,72] and by p90ATF6 decrease, but accompanied by accumulation of p50ATF6 in nuclear extracts [48,72]. The increase in intracellular

levels of calcium induced upon celecoxib treatment is most likely a trigger to ATF4 and ATF6 expression and GRP78 and ORP150 upregulation, since the addition of a chelating agent at least partially inhibited these celecoxib-induced events [48,72].

Besides celecoxib, other NSAIDs are able to modulate ER stress elements in cancer cells. S(+)-ibuprofen, the active stereoisomer of ibuprofen, was shown to up regulate various UPR related genes (like ATF4, ATF6 and XBP1) in a neuroblastoma cell line [73]. In 786-O renal carcinoma cells, indomethacin (200 μM) induced GRP78, GRP94, and CHOP protein expression and also ATF6 activation [74]. Niflumic acid (100 μM) was shown to induce eIF2alpha and PERK phosphorylation and CHOP expression in non-small cell lung cancer cell lines A549 and H460, effects that were intensified when this NSAID was combined with ciglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) ligand that has anti-tumor activity [75]. Intestinal cancer cells HCT-116 treated with 25 μM sulindac sulfide, the active metabolite of sulindac, presented increases in GRP78, CHOP, GADD34, ATF4 and ATF3 mRNA expression [76]. In C6 and glioma cells, 100 μM sulindac sulfide induced an increase in intracellular calcium concentration related to the release of calcium from intracellular stores, in a similar way to that observed for 100 μM celecoxib. Induction of GRP78 protein expression was observed in other glioma cell line, U87-MG, treated with sulindac sulfide or celecoxib, but CHOP protein expression was not induced by sulindac sulfide in these cells. The authors indicated with other experiments that the difference in CHOP expression can be related to the effect of each drug on different intracellular calcium stores: sulindac sulfide would only act on the ER, while celecoxib would induce calcium release from the ER and from mitochondria. Mitochondrial perturbation together with ER stress would then be necessary to induce CHOP expression in these cells [77].

2.2.1.1 Combined effects of NSAIDs and other compounds

Combination of celecoxib with other anti-cancer drugs might lead to ER stress aggravation, which can result in enhanced cell death because of the inability of cells to deal with the misfolded or unfolded proteins. Simultaneous treatment of glioblastoma cells with the proteasome inhibitor bortezomib and celecoxib, or bortezomib and DMC, resulted in increased cell death, in higher protein levels of CHOP and GRP78 than with each drug alone, and in the activation of JNK1/2 and caspases 3, 4, 7 and 9. Additionally, *in vivo* experiments with tumor-bearing mice showed that the combination of bortezomib and DMC resulted not only in higher CHOP expression, evidenced by immunohistochemistry studies, but also in increased apoptosis, demonstrated by a higher number of TUNEL positive cells [78].

MG132, another proteasome inhibitor, in combination with celecoxib led to decreased viability of urothelial carcinoma cells NTUB1 and T24 [70] and human liver tumor cells HepG2 and HA22T/VGH [79]. Moreover, in the urothelial carcinoma cells the combination of drugs induced higher caspases 3, 7, 8 and 9 activation, PARP cleavage, and increased expression of CHOP and GRP78 [70]. In the liver tumor cells, PARP cleavage was also present and higher expression of CHOP (in both cell lines) and ATF4 (only in HepG2 cells) mRNA and XBP1 mRNA processing were also induced by simultaneous treatment with MG132 and celecoxib [79].

In human breast carcinoma cell lines, in a combined therapy of a NSAID with nelfinavir (15-30 μM), an HIV protease inhibitor that acts as an ER stress aggravator through proteasome proteases inhibition, it was shown a reduction in cell survival and an increase in apoptosis. Interestingly, this was achieved by the combination with either celecoxib (40-50 μM) or DMC (30-40 μM), even in cells that are resistant to doxorubicin or taxol, or to other anti-cancer drugs that display different mechanisms of action. Combined treatment with DMC and nelfinavir also induced higher GRP78 and CHOP expression, caspase 7 activation and PARP cleavage and reduced colony formation. Silencing either of GRP78 or CHOP resulted in significantly altered chemosensitivity of tumor cells to the combined use of the compounds, as the blockage of GRP78 was associated with increased chemosensitization to the extent to that CHOP knock-down resulted in increased survival, thus establishing ER stress as a determinant event in this process [80].

Various critical intracellular signaling pathways were investigated in a study where a combination of celecoxib with phosphodiesterase 5 (PDE5) inhibitors was tested for the ability to kill multiple tumor cells [81]. The drug combination elicited ER stress response where eIF2α, ATF4 and CHOP seemed to be critical components to mediate tumor killing. However, IRE1 and XBP1 seemed to play a resistance role in the killing effects induced by the combination, as their knock down led to enhanced tumor killing. This data demonstrates that combination of celecoxib and specific PDE5 inhibitors such as sildenafil, at clinical relevant concentrations, have the potential to be a new therapeutic approach for cancer.

Autophagy is a cellular response that can be activated to generate ATP during starvation but that also functions

as a quality control mechanism for proteins and organelles in order to keep cell homeostasis. Autophagy has been implicated as a survival mechanism for cancer cells and new autophagy inhibitors are currently being studied [82,83]. Because of the protein quality control property, inhibition of autophagy can result in ER stress aggravation in a similar way to proteasome inhibitors. Indeed, in triple negative breast cancer cells the combination of chloroquine (20 μM) or mefloquine (10 μM) with DMC (20 μM) and nelfinavir (25 μM) induced higher GRP78 and CHOP expression, apoptosis, and lower colony formation than as reported for DMC and nelfinavir combination discussed above [84].

2.2.2 Metabolic diseases

An important study by Ozcan *et al.* suggested a link between obesity and ER stress, which could then lead to insulin resistance. This is especially important because in adipose tissue, and also in the liver from mice fed a high-fat diet, PERK and IRE1 are significantly phosphorylated [85]. Therefore, investigating the effects of NSAIDs as compounds with the ability to reduce ER stress in metabolic diseases can provide potential alternative therapy.

Flurbiprofen, but not aspirin, meloxicam, or ibuprofen, was shown to have strong chaperone activity, which resulted in lowering of protein aggregation *in vitro*. Further investigations demonstrated that flurbiprofen possesses the ability to attenuate the activation of all three ER stress branches, through lowering of XBP1 processing and CHOP and HERP expression induced by tunicamycin in a neuroblastoma cell line. Also, in a model of obese mice, the authors demonstrated that flurbiprofen attenuated leptin resistance and reduced body weight gain and visceral fat, effects that were not observed in aspirin, meloxicam or ibuprofen treated mice [86].

In an *in vitro* study, sodium salicylate, the metabolite of aspirin, has been shown to alleviate the ER stress by decreasing the expression of ER stress markers such as ATF6, CHOP, and GRP78 in cultured primary human adipocytes treated with lipopolysaccharide (LPS), high glucose concentration, saturated fatty acids, or the ER stress inducer tunicamycin [33]. Taken together, these studies indicate that some NSAIDs can display beneficial effects in metabolic diseases by relieving ER stress response.

2.2.3 Neuroprotection

Neurodegenerative protein misfolding disorders (PMDs), such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and prion related diseases involve the accumulation of misfolded proteins, but each of them has different clinical manifestations. In animal models of PMDs and in brain tissue from patients with PMD the accumulation of abnormal proteins has been linked to alterations in ER stress markers (reviewed in [87]). Increasing evidence suggests that the UPR could play a role in normal neuronal function as well as in the development of neurodegenerative diseases. Therefore, depending on the context specific for each disease, the same ER stress component could even have opposite roles (recently reviewed in [88]).

Yamazaki *et al.* [89] demonstrated that various NSAIDs (diclofenac, indomethacin, ibuprofen, aspirin, and ketoprofen) inhibit ER stress-induced cell death of human neuroblastoma SH-SY5Y cells. Cells were pre-treated with each drug for 2 hours and then thapsigargin or tunicamycin was added to the culture medium for 24 hours. Except for aspirin, that only inhibited tunicamycin-induced cell death, all other drugs inhibited cell death in different proportions, and diclofenac was the one with higher inhibition for both stressors.

In primary culture of glial cells, pranoprofen reduced GRP78 and CHOP mRNA and more slightly protein expression, when cells were pre-treated for 1 hour with this drug and later challenged with tunicamycin. Pranoprofen pre-treatment also induced eIF2alpha phosphorylation and inhibited XBP1 splicing, but did not affect IRE1 phosphorylation induced by tunicamycin [90].

In the context of cerebral ischemia, subcutaneous meloxicam (0,5 mg/Kg) reduced mRNA levels of CHOP, GRP78 and GRP94, which were highly increased after the induction of ischemic injury (carotid occlusion) followed by 48 hours reperfusion in various rat brain areas. Meloxicam also induced higher GRP78 protein expression in the cerebral cortex of control animals, but reduced GRP78 protein expression in two hippocampal regions of injured animals. In the other areas no differences in expression were observed [91]. Intraperitoneal parecoxib (10 mg/Kg or 30 mg/Kg) induced GRP78 and ORP150 protein expression in ischemic areas of rat brains in relation to vehicle treated animals. On the other hand, CHOP protein expression and nuclear translocation were suppressed by parecoxib [92].

3 Concluding remarks

From the evidence provided in the published articles, NSAIDs perturb ER homeostasis by eliciting hallmark events including mostly the expression of GRP78 and CHOP, and the activation of PERK and ATF6. Although XBP1 was demonstrated in many articles to have the expression induced by NSAIDs, only a few demonstrated NSAIDs to cause the activation of IRE-1. These observations implicate that two ER stress branches largely mediate the ER stress-mediated effects of NSAIDs in cells.

It is noteworthy that a handful of evidence is provided regarding either the activation or inhibition of ER stress components upon *in vitro* exposure of cells or *in vivo* administration of NSAIDs. Certainly, the effects of NSAIDs on the activity, expression or repression of ER stress components may present broad effects beyond cytotoxic and therapeutic ones, as we have addressed in this review. Therefore, the interpretation of these data must take into account that various cell types and tissues, mice background, drugs' biodistribution, and a range of distinct class of NSAIDs and concentrations have been used. Nonetheless, despite what we currently know about the ER stress components that are affected by non-steroidal anti-inflammatory drugs (NSAIDs) and how these drugs modulate the activity of those components, more detailed mechanistic studies are in need to provide solid knowledge on how such effects are initially triggered upon exposure of cells to these drugs, being one of the major challenges in this field. While is unclear whether NSAIDs cause unfolded protein accumulation in ER, it is known that these compounds can cause membrane aberrancy, therefore opening up important questions whether the mechanisms underlying the effects of NSAIDs on UPR signaling involves lipid perturbation. Finally, prevention and effective therapy for diseases such as cancer where NSAIDs can be a choice will likely require the combined use of anti-proliferative agents, and much likely yet, the modulation of more than one ER stress target molecule.

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References

[1] Bertolotti A., Zhang Y., Hendershot L.M., Harding H.P., Ron D., Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response, Nat Cell Biol, 2000, 2(6), 326-32.

[2] Shen J., Chen X., Hendershot L., Prywes R., ER stress regulation of ATF6 localization by dissociation of BiP/GRP78 binding and unmasking of Golgi localization signals, Dev Cell, 2002, 3(1), 99-111.

[3] Nadanaka S., Okada T., Yoshida H., Mori K., Role of disulfide bridges formed in the luminal domain of ATF6 in sensing endoplasmic reticulum stress, Mol Cell Biol, 2007, 27(3), 1027-43.

[4] Gardner B.M., Walter P., Unfolded proteins are Ire1-activating ligands that directly induce the unfolded protein response, Science, 2011, 333(6051), 1891-4.

[5] Volmer R., van der Ploeg K., Ron D., Membrane lipid saturation activates endoplasmic reticulum unfolded protein response transducers through their transmembrane domains, Proc Natl Acad Sci U S A, 2013, 110(12), 4628-33.

[6] Cunha D.A., Hekerman P., Ladriere L., Bazarra-Castro A., Ortis F., Wakeham M.C., et al., Initiation and execution of lipotoxic ER stress in pancreatic beta-cells, J Cell Sci, 2008, 121(Pt 14), 2308-18.

[7] Feng B., Yao P.M., Li Y., Devlin C.M., Zhang D., Harding H.P., et al., The endoplasmic reticulum is the site of cholesterol-induced cytotoxicity in macrophages, Nat Cell Biol, 2003, 5(9), 781-92.

[8] Calfon M., Zeng H., Urano F., Till J.H., Hubbard S.R., Harding H.P., et al., IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA, Nature, 2002, 415(6867), 92-6.

[9] Chen X., Shen J., Prywes R., The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi, J Biol Chem, 2002, 277(15), 13045-52.

[10] Haze K., Yoshida H., Yanagi H., Yura T., Mori K., Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress, Mol Biol Cell, 1999, 10(11), 3787-99.

[11] Ye J., Rawson R.B., Komuro R., Chen X., Dave U.P., Prywes R., et al., ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs, Mol Cell, 2000, 6(6), 1355-64.

[12] Kokame K., Kato H., Miyata T., Identification of ERSE-II, a new cis-acting element responsible for the ATF6-dependent mammalian unfolded protein response, J Biol Chem, 2001, 276(12), 9199-205.

[13] Yoshida H., Haze K., Yanagi H., Yura T., Mori K., Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors, J Biol Chem, 1998, 273(50), 33741-9.

[14] Yoshida H., Matsui T., Yamamoto A., Okada T., Mori K., XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor, Cell, 2001, 107(7), 881-91.

[15] Yoshida H., Okada T., Haze K., Yanagi H., Yura T., Negishi M., et al., Endoplasmic reticulum stress-induced formation of

transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response, Mol Cell Biol, 2001, 21(4), 1239-48.
[16] Sato Y., Nadanaka S., Okada T., Okawa K., Mori K., Luminal domain of ATF6 alone is sufficient for sensing endoplasmic reticulum stress and subsequent transport to the Golgi apparatus, Cell Struct Funct, 2011, 36(1), 35-47.
[17] Harding H.P., Zeng H., Zhang Y., Jungries R., Chung P., Plesken H., et al., Diabetes mellitus and exocrine pancreatic dysfunction in perk-/- mice reveals a role for translational control in secretory cell survival, Mol Cell, 2001, 7(6), 1153-63.
[18] Harding H.P., Zhang Y., Ron D., Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature, 1999, 397(6716), 271-4.
[19] Harding H.P., Zhang Y., Bertolotti A., Zeng H., Ron D., Perk is essential for translational regulation and cell survival during the unfolded protein response, Mol Cell, 2000, 5(5), 897-904.
[20] Harding H.P., Zhang Y., Zeng H., Novoa I., Lu P.D., Calfon M., et al., An integrated stress response regulates amino acid metabolism and resistance to oxidative stress, Mol Cell, 2003, 11(3), 619-33.
[21] Vattem K.M., Wek R.C., Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells, Proc Natl Acad Sci U S A, 2004, 101(31), 11269-74.
[22] McCullough K.D., Martindale J.L., Klotz L.O., Aw T.Y., Holbrook N.J., Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, Mol Cell Biol, 2001, 21(4), 1249-59.
[23] Brunton L.L., Chabner B.A., Knollman B.C., Goodman and Gilman's the pharmacological basis of therapeutics, 2011, 1808p.
[24] Bacchi S., Palumbo P., Sponta A., Coppolino M.F., Clinical pharmacology of non-steroidal anti-inflammatory drugs: a review, Antiinflamm Antiallergy Agents Med Chem, 2012, 11(1), 52-64.
[25] Abdel-Tawab M., Zettl H., Schubert-Zsilavecz M., Nonsteroidal anti-inflammatory drugs: a critical review on current concepts applied to reduce gastrointestinal toxicity, Curr Med Chem, 2009, 16(16), 2042-63.
[26] Chandrasekharan N.V., Dai H., Roos K.L., Evanson N.K., Tomsik J., Elton T.S., et al., COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression, Proc Natl Acad Sci U S A, 2002, 99(21), 13926-31.
[27] Kis B., Snipes J.A., Busija D.W., Acetaminophen and the cyclooxygenase-3 puzzle: sorting out facts, fictions, and uncertainties, J Pharmacol Exp Ther, 2005, 315(1), 1-7.
[28] Lee Y.S., Kim H., Brahim J.S., Rowan J., Lee G., Dionne R.A., Acetaminophen selectively suppresses peripheral prostaglandin E2 release and increases COX-2 gene expression in a clinical model of acute inflammation, Pain, 2007, 129(3), 279-86.
[29] Hinz B., Cheremina O., Brune K., Acetaminophen (paracetamol) is a selective cyclooxygenase-2 inhibitor in man, Faseb J, 2008, 22(2), 383-90.
[30] Stone E., An account of the success of the bark of the willow tree in the cure of agues. In a letter to the high honourable George Earl of Macclesfield, President of R. S. from the Rev. Mr. Edmund Stone, of Chipping-Norton in Oxfordshire., Philosophical Transactions, 1763, 53.
[31] Amann R., Peskar B.A., Anti-inflammatory effects of aspirin and sodium salicylate, Eur J Pharmacol, 2002, 447(1), 1-9.
[32] Vainio H., Morgan G., Aspirin for the second hundred years: new uses for an old drug, Pharmacol Toxicol, 1997, 81(4), 151-2.
[33] Alhusaini S., McGee K., Schisano B., Harte A., McTernan P., Kumar S., et al., Lipopolysaccharide, high glucose and saturated fatty acids induce endoplasmic reticulum stress in cultured primary human adipocytes: Salicylate alleviates this stress, Biochem Biophys Res Commun, 2010, 397(3), 472-8.
[34] Silva A.M., Wang D., Komar A.A., Castilho B.A., Williams B.R., Salicylates trigger protein synthesis inhibition in a protein kinase R-like endoplasmic reticulum kinase-dependent manner, J Biol Chem, 2007, 282(14), 10164-71.
[35] Gentz S.H., Bertollo C.M., Souza-Fagundes E.M., da Silva A.M., Implication of eIF2alpha kinase GCN2 in induction of apoptosis and endoplasmic reticulum stress-responsive genes by sodium salicylate, J Pharm Pharmacol, 2013, 65(3), 430-40.
[36] Mehta D., Bhargava D.K., Non-steroidal anti inflammatory drugs and gastrointestinal toxicity, Apollo Medicine, 2010, 7(4), 251-62.
[37] Boelsterli U.A., Redinbo M.R., Saitta K.S., Multiple NSAID-induced hits injure the small intestine: underlying mechanisms and novel strategies, Toxicol Sci, 2012, 131(2), 654-67.
[38] Wallace J.L., McKnight W., Reuter B.K., Vergnolle N., NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2, Gastroenterology, 2000, 119(3), 706-14.
[39] Langenbach R., Morham S.G., Tiano H.F., Loftin C.D., Ghanayem B.I., Chulada P.C., et al., Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration, Cell, 1995, 83(3), 483-92.
[40] Tomisato W., Tsutsumi S., Hoshino T., Hwang H.J., Mio M., Tsuchiya T., et al., Role of direct cytotoxic effects of NSAIDs in the induction of gastric lesions, Biochem Pharmacol, 2004, 67(3), 575-85.
[41] Tomisato W., Tsutsumi S., Rokutan K., Tsuchiya T., Mizushima T., NSAIDs induce both necrosis and apoptosis in guinea pig gastric mucosal cells in primary culture, Am J Physiol Gastrointest Liver Physiol, 2001, 281(4), G1092-100.
[42] Tsutsumi S., Gotoh T., Tomisato W., Mima S., Hoshino T., Hwang H.J., et al., Endoplasmic reticulum stress response is involved in nonsteroidal anti-inflammatory drug-induced apoptosis, Cell Death Differ, 2004, 11(9), 1009-16.
[43] Ohyama K., Shiokawa A., Ito K., Masuyama R., Ichibangase T., Kishikawa N., et al., Toxicoproteomic analysis of a mouse model of nonsteroidal anti-inflammatory drug-induced gastric ulcers, Biochem Biophys Res Commun, 2012, 420(1), 210-5.
[44] Pereira-Leite C., Nunes C., Reis S., Interaction of nonsteroidal anti-inflammatory drugs with membranes: in vitro assessment and relevance for their biological actions, Prog Lipid Res, 2013, 52(4), 571-84.
[45] Tanaka K., Tomisato W., Hoshino T., Ishihara T., Namba T., Aburaya M., et al., Involvement of intracellular Ca2+ levels in nonsteroidal anti-inflammatory drug-induced apoptosis, J Biol Chem, 2005, 280(35), 31059-67.
[46] Ishihara T., Hoshino T., Namba T., Tanaka K., Mizushima T., Involvement of up-regulation of PUMA in non-steroidal

anti-inflammatory drug-induced apoptosis, Biochem Biophys Res Commun, 2007, 356(3), 711-7.
[47] Pyrko P., Kardosh A., Schonthal A.H., Celecoxib transiently inhibits cellular protein synthesis, Biochem Pharmacol, 2008, 75(2), 395-404.
[48] Tsutsumi S., Namba T., Tanaka K.I., Arai Y., Ishihara T., Aburaya M., et al., Celecoxib upregulates endoplasmic reticulum chaperones that inhibit celecoxib-induced apoptosis in human gastric cells, Oncogene, 2006, 25(7), 1018-29.
[49] Namba T., Hoshino T., Suemasu S., Takarada-Iemata M., Hori O., Nakagata N., et al., Suppression of expression of endoplasmic reticulum chaperones by Helicobacter pylori and its role in exacerbation of non-steroidal anti-inflammatory drug-induced gastric lesions, J Biol Chem, 2010, 285(48), 37302-13.
[50] Namba T., Hoshino T., Tanaka K., Tsutsumi S., Ishihara T., Mima S., et al., Up-regulation of 150-kDa oxygen-regulated protein by celecoxib in human gastric carcinoma cells, Mol Pharmacol, 2007, 71(3), 860-70.
[51] Teoh N.C., Farrell G.C., Hepatotoxicity associated with non-steroidal anti-inflammatory drugs, Clin Liver Dis, 2003, 7(2), 401-13.
[52] Unzueta A., Vargas H.E., Nonsteroidal anti-inflammatory drug-induced hepatoxicity, Clin Liver Dis, 2013, 17(4), 643-56, ix.
[53] Nadanaciva S., Aleo M.D., Strock C.J., Stedman D.B., Wang H., Will Y., Toxicity assessments of nonsteroidal anti-inflammatory drugs in isolated mitochondria, rat hepatocytes, and zebrafish show good concordance across chemical classes, Toxicol Appl Pharmacol, 2013, 272(2), 272-80.
[54] Franceschelli S., Moltedo O., Amodio G., Tajana G., Remondelli P., In the Huh7 Hepatoma Cells Diclofenac and Indomethacin Activate Differently the Unfolded Protein Response and Induce ER Stress Apoptosis, Open Biochem J, 2011, 5, 45-51.
[55] El-Hassan H., Anwar K., Macanas-Pirard P., Crabtree M., Chow S.C., Johnson V.L., et al., Involvement of mitochondria in acetaminophen-induced apoptosis and hepatic injury: roles of cytochrome c, Bax, Bid, and caspases, Toxicol Appl Pharmacol, 2003, 191(2), 118-29.
[56] Nagy G., Kardon T., Wunderlich L., Szarka A., Kiss A., Schaff Z., et al., Acetaminophen induces ER dependent signaling in mouse liver, Arch Biochem Biophys, 2007, 459(2), 273-9.
[57] Hur K.Y., So J.S., Ruda V., Frank-Kamenetsky M., Fitzgerald K., Koteliansky V., et al., IRE1alpha activation protects mice against acetaminophen-induced hepatotoxicity, J Exp Med, 2012, 209(2), 307-18.
[58] Uzi D., Barda L., Scaiewicz V., Mills M., Mueller T., Gonzalez-Rodriguez A., et al., CHOP is a critical regulator of acetaminophen-induced hepatotoxicity, J Hepatol, 2013, 59(3), 495-503.
[59] Lorz C., Justo P., Sanz A., Subira D., Egido J., Ortiz A., Paracetamol-induced renal tubular injury: a role for ER stress, J Am Soc Nephrol, 2004, 15(2), 380-9.
[60] Kalinec G.M., Thein P., Parsa A., Yorgason J., Luxford W., Urrutia R., et al., Acetaminophen and NAPQI are toxic to auditory cells via oxidative and endoplasmic reticulum stress-dependent pathways, Hear Res, 2014, 313, 26-37.
[61] Grosch S., Maier T.J., Schiffmann S., Geisslinger G., Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors, J Natl Cancer Inst, 2006, 98(11), 736-47.
[62] Koki A.T., Masferrer J.L., Celecoxib: a specific COX-2 inhibitor with anticancer properties, Cancer Control, 2002, 9(2 Suppl), 28-35.
[63] Liggett J.L., Zhang X., Eling T.E., Baek S.J., Anti-tumor activity of non-steroidal anti-inflammatory drugs: cyclooxygenase-independent targets, Cancer Lett, 2014, 346(2), 217-24.
[64] Chuang H.C., Kardosh A., Gaffney K.J., Petasis N.A., Schonthal A.H., COX-2 inhibition is neither necessary nor sufficient for celecoxib to suppress tumor cell proliferation and focus formation in vitro, Mol Cancer, 2008, 7, 38.
[65] Chen S.T., Thomas S., Gaffney K.J., Louie S.G., Petasis N.A., Schonthal A.H., Cytotoxic effects of celecoxib on Raji lymphoma cells correlate with aggravated endoplasmic reticulum stress but not with inhibition of cyclooxygenase-2, Leuk Res, 2010, 34(2), 250-3.
[66] Johnson A.J., Hsu A.L., Lin H.P., Song X., Chen C.S., The cyclo-oxygenase-2 inhibitor celecoxib perturbs intracellular calcium by inhibiting endoplasmic reticulum Ca2+-ATPases: a plausible link with its anti-tumour effect and cardiovascular risks, Biochem J, 2002, 366(Pt 3), 831-7.
[67] Cha W., Park S.W., Kwon T.K., Hah J.H., Sung M.W., Endoplasmic reticulum stress response as a possible mechanism of cyclooxygenase-2-independent anticancer effect of celecoxib, Anticancer Res, 2014, 34(4), 1731-5.
[68] Pyrko P., Kardosh A., Liu Y.T., Soriano N., Xiong W., Chow R.H., et al., Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2,5-dimethyl-celecoxib, a non-coxib analogue of celecoxib, Mol Cancer Ther, 2007, 6(4), 1262-75.
[69] Du H., Li W., Wang Y., Chen S., Zhang Y., Celecoxib induces cell apoptosis coupled with up-regulation of the expression of VEGF by a mechanism involving ER stress in human colorectal cancer cells, Oncol Rep, 2011, 26(2), 495-502.
[70] Huang K.H., Kuo K.L., Chen S.C., Weng T.I., Chuang Y.T., Tsai Y.C., et al., Down-regulation of glucose-regulated protein (GRP) 78 potentiates cytotoxic effect of celecoxib in human urothelial carcinoma cells, PLoS One, 2012, 7(3), e33615.
[71] Zhang X., Lee S.H., Min K.W., McEntee M.F., Jeong J.B., Li Q., et al., The involvement of endoplasmic reticulum stress in the suppression of colorectal tumorigenesis by tolfenamic acid, Cancer Prev Res (Phila), 2013, 6(12), 1337-47.
[72] Namba T., Ishihara T., Tanaka K., Hoshino T., Mizushima T., Transcriptional activation of ATF6 by endoplasmic reticulum stressors, Biochem Biophys Res Commun, 2007, 355(2), 543-8.
[73] Ikegaki N., Hicks S.L., Regan P.L., Jacobs J., Jumbo A.S., Leonhardt P., et al., S(+)-ibuprofen destabilizes MYC/MYCN and AKT, increases p53 expression, and induces unfolded protein response and favorable phenotype in neuroblastoma cell lines, Int J Oncol, 2014, 44(1), 35-43.
[74] Ou Y.C., Yang C.R., Cheng C.L., Raung S.L., Hung Y.Y., Chen C.J., Indomethacin induces apoptosis in 786-O renal cell carcinoma cells by activating mitogen-activated protein kinases and AKT, Eur J Pharmacol, 2007, 563(1-3), 49-60.
[75] Kim B.M., Maeng K., Lee K.H., Hong S.H., Combined treatment with the Cox-2 inhibitor niflumic acid and PPARgamma ligand ciglitazone induces ER stress/caspase-8-mediated apoptosis in human lung cancer cells, Cancer Lett, 2011, 300(2), 134-44.
[76] Yang H., Park S.H., Choi H.J., Moon Y., The integrated stress response-associated signals modulates intestinal tumor cell

growth by NSAID-activated gene 1 (NAG-1/MIC-1/PTGF-beta), Carcinogenesis, 2010, 31(4), 703-11.
[77] White M.C., Johnson G.G., Zhang W., Hobrath J.V., Piazza G.A., Grimaldi M., Sulindac sulfide inhibits sarcoendoplasmic reticulum Ca2+ ATPase, induces endoplasmic reticulum stress response, and exerts toxicity in glioma cells: relevant similarities to and important differences from celecoxib, J Neurosci Res, 2013, 91(3), 393-406.
[78] Kardosh A., Golden E.B., Pyrko P., Uddin J., Hofman F.M., Chen T.C., et al., Aggravated endoplasmic reticulum stress as a basis for enhanced glioblastoma cell killing by bortezomib in combination with celecoxib or its non-coxib analogue, 2,5-dimethyl-celecoxib, Cancer Res, 2008, 68(3), 843-51.
[79] Cusimano A., Azzolina A., Iovanna J.L., Bachvarov D., McCubrey J.A., D'Alessandro N., et al., Novel combination of celecoxib and proteasome inhibitor MG132 provides synergistic antiproliferative and proapoptotic effects in human liver tumor cells, Cell Cycle, 2010, 9(7), 1399-410.
[80] Cho H.Y., Thomas S., Golden E.B., Gaffney K.J., Hofman F.M., Chen T.C., et al., Enhanced killing of chemo-resistant breast cancer cells via controlled aggravation of ER stress, Cancer Lett, 2009, 282(1), 87-97.
[81] Booth L., Roberts J.L., Cruickshanks N., Tavallai S., Webb T., Samuel P., et al., PDE5 inhibitors enhance Celecoxib killing in multiple tumor types, J Cell Physiol, 2014.
[82] Amaravadi R.K., Thompson C.B., The roles of therapy-induced autophagy and necrosis in cancer treatment, Clin Cancer Res, 2007, 13(24), 7271-9.
[83] Jin S., White E., Role of autophagy in cancer: management of metabolic stress, Autophagy, 2007, 3(1), 28-31.
[84] Thomas S., Sharma N., Golden E.B., Cho H., Agarwal P., Gaffney K.J., et al., Preferential killing of triple-negative breast cancer cells in vitro and in vivo when pharmacological aggravators of endoplasmic reticulum stress are combined with autophagy inhibitors, Cancer Lett, 2012, 325(1), 63-71.
[85] Ozcan U., Cao Q., Yilmaz E., Lee A.H., Iwakoshi N.N., Ozdelen E., et al., Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science, 2004, 306(5695), 457-61.
[86] Hosoi T., Yamaguchi R., Noji K., Matsuo S., Baba S., Toyoda K., et al., Flurbiprofen ameliorated obesity by attenuating leptin resistance induced by endoplasmic reticulum stress, EMBO Mol Med, 2014, 6(3), 335-46.
[87] Matus S., Glimcher L.H., Hetz C., Protein folding stress in neurodegenerative diseases: a glimpse into the ER, Curr Opin Cell Biol, 2011, 23(2), 239-52.
[88] Hetz C., Mollereau B., Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases, Nat Rev Neurosci, 2014, 15(4), 233-49.
[89] Yamazaki T., Muramoto M., Oe T., Morikawa N., Okitsu O., Nagashima T., et al., Diclofenac, a non-steroidal anti-inflammatory drug, suppresses apoptosis induced by endoplasmic reticulum stresses by inhibiting caspase signaling, Neuropharmacology, 2006, 50(5), 558-67.
[90] Hosoi T., Sasaki M., Baba S., Ozawa K., Effect of pranoprofen on endoplasmic reticulum stress in the primary cultured glial cells, Neurochem Int, 2009, 54(1), 1-6.
[91] Llorente I.L., Burgin T.C., Perez-Rodriguez D., Martinez-Villayandre B., Perez-Garcia C.C., Fernandez-Lopez A., Unfolded protein response to global ischemia following 48 h of reperfusion in the rat brain: the effect of age and meloxicam, J Neurochem, 2013, 127(5), 701-10.
[92] Ye Z., Wang N., Xia P., Wang E., Liao J., Guo Q., Parecoxib suppresses CHOP and Foxo1 nuclear translocation, but increases GRP78 levels in a rat model of focal ischemia, Neurochem Res, 2013, 38(4), 686-93.