Chapter 15
Sulforaphane-Loaded Nanomedicines Applications: Trends on Inflammatory Diseases and Cancer Treatment

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Abstract Sulforaphane (SFN), a natural isothiocyanate derivative, has been extensively studied as therapeutic compound. Different cellular pathways were described for explaining its promising pharmacological effects such as anti-inflammatory, antitumoral, and antioxidant. In this sense, several studies have investigated SFN as single or in association with conventional drugs, specially as anti-inflammatory and antitumoral. In this sense, new strategies for delivering SFN have been discussed for overcoming physicochemical and/or biopharmaceutics limitations by using a variety of nanocarriers types such as micelles, polymeric/lipid/inorganic nanoparticles, nanocomposites, and gels. In this chapter, a discussion associating SFN molecular mechanisms of action with its potential pharmacological applications and the main nanocarriers for SFN delivery are provided, highlighting the relationships between biological synthesis, pharmacological aspects, and the new nanotechnological strategies for developing effective and safe pharmacotherapeutic alternatives.

Keywords Sulforaphane · Nanomedicines · Inflammation · Cancer

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D. Ribeiro de Araujo, M. Carneiro-Ramos (eds.), *Biotechnology Applied to Inflammatory Diseases*, Interdisciplinary Biotechnological Advances,
https://doi.org/10.1007/978-981-19-8342-9_15

Abbreviations

ARE	Antioxidant response element
CSCs	Cancer stem-like cells
EOC	Epithelial ovarian cancer cell
ER	Estrogen receptor
GLS	Glycosinolates
GRR	Glycorafanine
GSH	Glutathione
GST	Glutathione S-transferase
HO-1	Heme-oxygenase-1
ICT	Isothiocyanates
MMPs	Metalloproteinases
NfkB	Nuclear factor kappa B
NQO1	Quinone oxidoreductase 1
Nrf2	Nuclear factor erythroid 2-related factor 2
NSCLCs	Non-small cell lung cancers cells
PC-3	Human prostate cancer cells in culture
PC-3	Human prostate cancer cells in culture
ROS	Reactive oxygen species
SFN	Sulforaphane
TNBC	Triple-negative breast cancer
γGCL	γ-Glutamylcysteine ligase

15.1 Sulforaphane: Biological Synthesis and Metabolism

Sulforaphane (SFN) [1-isothiocyanate-(4*R*)-(methylsulfinyl) butane] (Fig. 15.1a) is a natural compound widely studied since 1980 (Guerrero-Beltrán et al. 2012). It belongs to the group of isothiocyanates (ICT) phytochemicals and is found in abundance in cruciferous vegetables. These plants belong to the *Brassicaceae* family, which has about 350 genera and 3200 species, including broccoli (*Brassica oleracea var. italica*), white cabbage (*Brassica oleracea var. capitata*), cauliflower (*Brassica oleracea var. Botrytis*), Brussels sprouts (*Brassica oleracea var. gemmifera*), watercress (*Nasturtium officinalis*), white mustard (*Sinapis alba*), arugula (*Eruca sativa*), and radish (*Raphanus sativus*) (Fahey et al. 2001, 2015). Among them, broccoli and, in particular, its sprouts, have the greatest potential for extracting SFN (Totušek et al. 2011).

In fresh vegetables, SFN is obtained from the hydrolysis of glycorafanine (GRR), a secondary metabolite of glycosinolates (GLS) family, also called sulforaphane glycosinolate, from the catalytic activity of the enzyme myrosinase (Pérez et al. 2014). When vegetable tissues are processed by cutting, cooking, freezing, or chewing, GLS are exposed to the action of the enzyme myrosinase, which

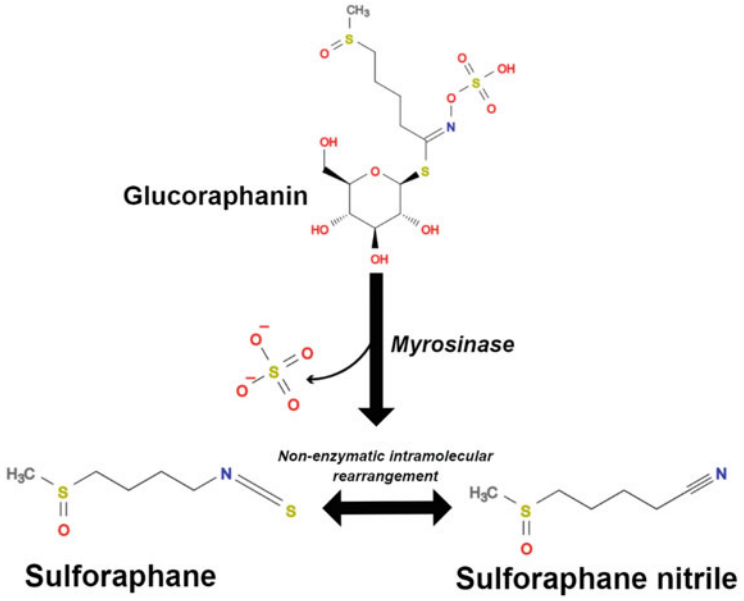

Fig. 15.1 Sulforaphane biological synthesis reaction

hydrolyzes them to isothiocyanates, which are the bioactive compounds (Fig. 15.1). The β-thioglucoside bond is hydrolyzed by myrosinase, producing glucose, sulfate, and a diverse group of aglycone products. The resultant aglycones then undergo nonenzymatic, intramolecular rearrangement to yield isothiocyanates, thiocyanates, or nitriles (Fig. 15.1).

In addition, the human intestinal flora is also capable of converting GLS into isothiocyanates with biological activity, as it has an isoform of the enzyme myrosinase, but hydrolysis in the intestinal tract manages to convert only between 14 and 20% of glucoraphanin in sulforaphane (Fahey et al. 2001; Rungapamestry et al. 2007; Van Eylen et al. 2007).

Some factors, such as basic pH and high temperatures, favor the formation of SFN from GRR, while acidic pH, the presence of ferrous ions and proteins (non-catalytic co-factors of the enzyme myrosinase) increase the nitrile formation of SFN which has no potential activity. However, the main determinant for isothiocyanates production from their precursor GLS is the way the vegetable is cooked. In this sense, the consumption of lightly cooked vegetables over overcooked vegetables is preferable. Additionally, the composition of the meal does not seem to alter the bioavailability of the SFN (Rungapamestry et al. 2007; Williams et al. 2008).

Broccoli is recognized as the best source of SFN, a portion can contain up to 60 mg of the precursor GRR (Rungapamestry et al. 2007). The ideal cooking condition that maximizes the SFN content in broccoli was determined by Pérez et al. (2014) and corresponds to immersion in water at 57 °C for 13 min. In this

condition, the minimum content of GLS and GRR was observed and the mirosinase showed its maximum activity. Fresh young broccoli sprouts contain 128 mg of GLS per gram of fresh weight, in contrast, blanched broccoli contained only 92 mg, cooked broccoli contained 47 mg, and frozen broccoli contained 45 mg per gram of fresh weight (Cieślik et al. 2007; Vanduchova et al. 2019). The determination of SFN from plant tissues or functional foods is based mainly on analysis by high-performance liquid chromatography (Vanduchova et al. 2019).

After ingestion, SFN is formed inside the gastrointestinal tract reversibly binding to thiols, organosulfur compounds that contain a group –SH. Then, they are transported by plasma proteins to cross the plasma membrane, by passive diffusion, entering tissue cells. After internalization, the ITCs will react with glutathione (GSH), forming its conjugate (*S*-(*N*-alkyl/arylthiocarbamyl)-glutathione), this reaction is catalyzed by the enzyme glutathione S-transferase (GST). The glutathione conjugate is released to the outside of cells through carrier proteins or MRPs "multidrug resistance proteins." In the middle extracellular, glutathione conjugated to γ-glutamyl and glycine residues, will be cleaved by the enzyme γ-glutamyl transferase (γ-GT) and dipeptidase giving rise to a cysteine conjugate that will be transported to the liver. Finally, the conjugate of cysteine, under the action of the enzyme N-acetyl transferase, will become mercapturic acid (Yagishita et al. 2019; Langston-Cox et al. 2020). After the formation of mercapturic acid, it is then transported to the kidney, where it will be eliminated (Yagishita et al. 2019; Langston-Cox et al. 2020).

15.2 Cellular and Molecular Mechanisms of Action

In the last years, the interest in extraction, isolation, and characterization of the biological activity of compounds from broccoli have been demonstrated by several published works, with the majority of studies dedicated to the analysis of GLS and related compounds, especially SFN (Singh and Singh 2012; Gupta et al. 2014; Mishra et al. 2019).

SFN cell signaling pathways are dependent on different molecular targets; however, their best-described mechanism of action is via the Nrf2 pathway (Kensler et al. 2012; Wu et al. 2019; Yagishita et al. 2019; Yang et al. 2020) (Fig. 15.2). Nrf2 is a central transcription factor with a central role on cellular redox process. In unstimulated cells, it is repressed by the protein Keap1, which causes the ubiquitination and subsequent degradation of Nrf2. SFN can interact with the Keap1 protein, disrupting the Nrf2–Keap1 interaction, allowing the nuclear activation and translocation of Nrf2. In the nucleus, Nrf2 binds to the antioxidant response element (ARE), a DNA region that promotes genes encoding antioxidant enzymes, including NAD (P) H: quinone oxidoreductase 1 (NQO1), heme-oxygenase-1 (HO-1), γ-glutamylcysteine ligase (γGCL), and thioredoxin (Vomhof-DeKrey and Picklo 2012) (Fig. 15.2).

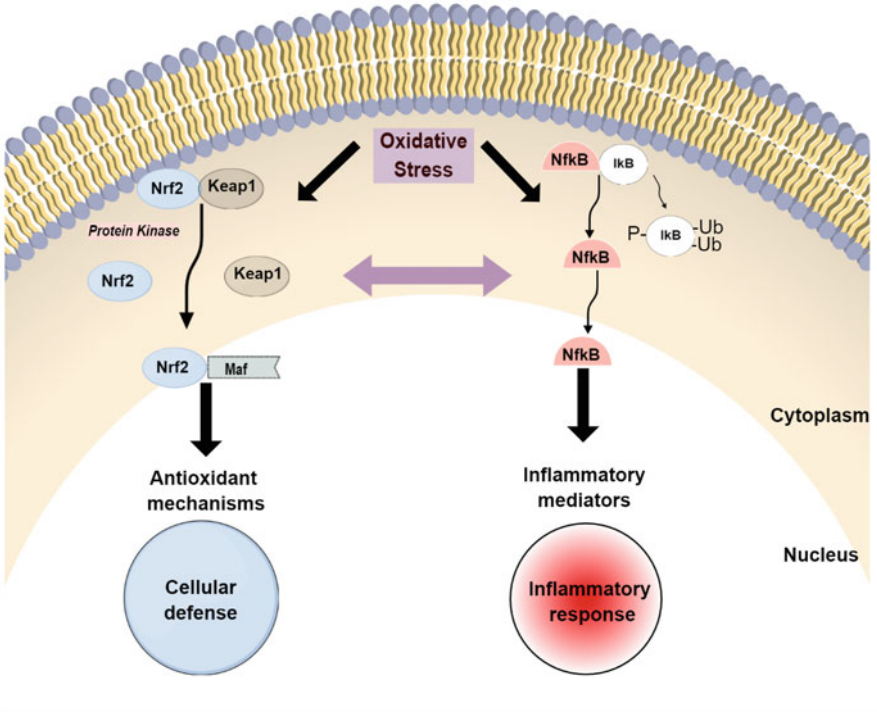

Fig. 15.2 Proposed molecular mechanism for sulforaphane anti-inflammatory activity through the NF-κB pathway

The enhanced transcription of Nrf2 target genes causes a strong cytoprotective response, increasing resistance to carcinogenesis and other diseases that have oxidative stress involved in pathogenesis, including neurodegenerative and chronic inflammatory diseases, such as colitis, atopic dermatitis, osteoarthritis (Nascimento et al. 2021; Kensler et al. 2012). In addition, SFN through the activation of Nrf2 increases the activity of phase II enzymes such as glutathione-S transferase (GST), involved in the elimination of xenobiotic compounds (Guerrero-Beltrán et al. 2012). It is suggested that the induction of phase II enzymes may be one of the main mechanisms by which cruciferous vegetables result in health benefits (Manchali et al. 2012).

Recently, several studies have shown that the SFN also has an anti-inflammatory activity, acting through the NF-κB pathway (Fig. 15.2).

The main mechanisms involved in the regulation of NF-κB signaling by SFN compresses the inhibition of phosphorylation and/or degradation of IkB, phosphorylation of IKK, and nuclear translocation of NF-κB (Fig. 15.2). All these mechanisms are described in the literature, in different cell types (Xu et al. 2005; Kim et al. 2012; Davidson et al. 2013, 2017). In a study using macrophages (cell line RAW 264.7), lipopolysaccharide-induced inflammation (LPS) was attenuated with SFN,

which negatively regulated the activity of the enzymes iNOS, COX-2, and the expression TNF-α (Heiss et al. 2001). Likewise, SFN reduced the synthesis of inflammatory mediators, such as interleukin IL-1β, TNF-α, and IL-6, induced by LPS, in primary microglial and rat astroglial cell co-cultures (Wierinckx et al. 2005). SFN also has anti-arthritic and immunoregulatory activity thus inhibiting synovial hyperplasia and the proliferation of activated T cells (Kong et al. 2010). In addition, it inhibits the expression of metalloproteinases (MMPs), as well as regulates the cell cycle keeping it in the G2/M phase, blocking joint degeneration both in vitro and in vivo (Heiss et al. 2001; Kim et al. 2009; Davidson et al. 2013, 2017).

15.2.1 Therapeutic Applications

This section presents and discusses relevant publications based on the progress in the design of SFN protective effect in a variety of in vivo pathologies as well as in in vitro studies on in vitro/in vivo experimental models, as summarized in Table 15.1.

The consumption of isothiocyanates, especially SFN, through the diet is directly related to the decreased risk of certain types of cancer, including lung, pancreas, ovarian, breast, prostate, colon, and bladder. It can act on multiple pathways: inhibiting growth, and proliferation of cancer cells, inducing apoptosis, inhibiting angiogenesis, and cell cycle as well as metastasis formation (Gupta et al. 2014; Kamal et al. 2020). In addition to acting as a chemopreventive, it also can act as an antineoplastic treatment (Singh and Singh 2012; Aumeeruddy and Mahomoodally 2019; Kamal et al. 2020).

Singh et al. (2005) demonstrate that SFN inhibited the growth of human prostate cancer cells in culture (PC-3), through the administration of 20 μM for 24 h, inducing apoptosis initiated by reactive oxygen species (ROS) generation (Singh et al. 2005). Similarly, Choi et al. (2007) also demonstrate the SFN effect on PC-3 and LNCaP prostate cancer cell lines. The in vitro treatment promoted the inactivation of inhibitors of apoptosis proteins (IAP-family) (Choi et al. 2007).

The effects of SFN treatment have also been evaluated in human bladder cancer T24 cell (Shan et al. 2006). Treatment with 10–40 μM SFN for 24 and 48 h significantly inhibited proliferation in a dose-dependent manner and also induced early apoptosis of T24 cell in a lower level of (5 μM) treatment (Shan et al. 2006).

SFN also inhibited cell growth and death in several human breast cancer cell lines, representative of a wide range of breast tumor phenotypes (MDA-MB231, MDA-MB-468, MCF-7, and T47D cells), by the inhibition of estrogen receptor (ER), EGFR1 and HER2, which are particularly important for the growth of breast cancer (Pledgie-Tracy et al. 2007). Another approach studied the effect of SFN on the inhibition of growth in breast ductal carcinoma (ZR-75-1) cells (Cheng et al. 2019). They demonstrated a cell cycle arrest (G1/S) caused by the downregulation of SERTAD1 gene expression by reducing the CDK4 activity in breast cancer cells (Cheng et al. 2019). Other in vitro and in vivo recent investigations reveal that SFN

Table 15.1 Sulforaphane anticancer and anti-inflammatory cellular and molecular mechanisms described in in vitro and in vivo models

Organ/tissue	Pathology	Cellular and molecular mechanisms	References
Bladder	Cancer	Inhibited the proliferation and induced apoptosis of T24 cells in vitro	Shan et al. (2006)
Breast	Cancer	Inhibition of cell growth (G2-M cell cycle block) and induction of apoptosis in multiple breast cancer cell lines	Pledgie-Tracy et al. (2007)
Breast	Cancer	SFN-paclitaxel-induced apoptosis by inhibiting the overexpression of Bcl-2	Kim et al. (2017)
Breast	Cancer	SFN enhanced the efficacy of doxorubicin in suppressing breast tumor growth	Bose et al. (2018)
Breast	Cancer	Inhibited the proliferation by G1/S arrest in breast carcinoma (ZR-75-1) cells	Cheng et al. (2019)
Breast	Cancer	Triple-negative breast cancer (TNBC) proliferation was suppressed in in vitro and in vivo models	Castro et al. (2019)
Colon	Cancer	Induction of G1-phase cell cycle arrest in HT-29 cells	Shen et al. (2006)
Colon	Cancer	Synergistic cytotoxicity effect with curcumin and dihydrocaffeic acid	Santana-Gálvez et al. (2020)
Digestive	Cancer	Suppression of migration and cell invasion in oral carcinoma	Jee et al. (2011)
Lung	Cancer	Arrest of cell migration and invasion avoiding metastasis of lung cancer	Wang et al. (2017)
Lymphoblastic leukemia	Cancer	Inhibition of lymphoblastic leukemia, inducing cell cycle arrest	Suppipat et al. (2012)
Ovarian	Cancer	SFN induces growth arrest and apoptosis epithelial ovarian cancer cell (EOC) line	Bryant et al. (2010)
Ovarian	Cancer	Inhibition of ovarian cancer progression via cell cycle and apoptosis	Kan et al. (2018)
Pancreas	Cancer	Inhibited human pancreatic carcinogenesis, reducing proliferation and tissue invasion	Li et al. (2013)
Prostate	Cancer	Induced apoptosis in PC-3 cells by ROS generation	Singh et al. (2005)
Prostate	Cancer	Inactivation of inhibitors of apoptosis inducing the death of human prostate cancer cells	Choi et al. (2007)
Cartilage	Rheumatoid arthritis	Pro-inflammatory cytokines reduction and synovial hyperplasia in vitro and in vivo models	Kong et al. (2010)
Cartilage	Osteoarthritis/ rheumatoid arthritis	Inhibition of cytokine-induced metalloproteinase expression in human chondrocytes and synovial cells	Davidson et al. (2013)
Cartilage	Osteoarthritis	SFN-rich diet can provide chondroprotection	Davidson et al. (2017)

(continued)

Table 15.1 (continued)

Organ/tissue	Pathology	Cellular and molecular mechanisms	References
Skin	Atopic dermatitis	Inhibition of IFN-γ and TNF-α-induced production of TARC/CCL17 and MDC/CCL22 in human HaCaT cells by inhibition of NF-κB pathway	Jeong et al. (2010)
Skin	Skin inflammation	Reduced inflammation scores in atopic dermatitis mice model	Wu et al. (2019)

can inhibit malignant cell proliferation and tumor sphere formation of cancer stem-like cells (CSCs) in triple-negative breast cancer (TNBC) model (Castro et al. 2019).

Some studies have shown SFN to be effective in preventing ovarian cancer, another important gynecologic cancer-associated mortality. Kan et al. (2018) investigation indicated that SFN effectively suppressed ovarian cancer cells (A2780 and OVCAR lines) proliferation, migration, and cell cycle progression, and also enhance apoptosis (Kan et al. 2018). SFN also inhibited the growth of epithelial ovarian cancer cell (EOC) (MDAH-2774 and SkOV-3 line) in vitro by the modulation of cell cycle regulatory proteins and by increasing apoptosis (Bryant et al. 2010).

SFN was able to regulate the cell cycle and inhibit its proliferation in other types of cancer. Suppipat et al. (2012) investigated in vitro the SFN activity in lymphoblastic leukemia cells, noting that after exposure of 15 μM for 1 day, these cells undergo cell cycle arrest and apoptosis thus preventing their multiplication and invasion to other tissues (Suppipat et al. 2012). Shen et al. (2006) detected the antiproliferative effects of SFN in the human colon carcinoma cell line, HT-29, by blocking the cell cycle at G1 (Shen et al. 2006).

As another important feature, SFN also induces anti-metastatic effects by suppressing cell migration and invasion. Li et al. (2013) studied the hypothesis of SFN acting on the malignant cells of pancreas in vivo (Li et al. 2013). Having verified, that with the administration of a dose between 0–20 mg/kg in mice over a 6-week period, the cell carcinogens were suppressed. SFN also inhibited cell migration and invasion through blockade of miR-616-5p expression and suppression of the epithelial-mesenchymal transition (EMT) process in non-small cell lung cancers (NSCLCs) cells (Wang et al. 2017). Jee et al. (2011) demonstrated that the anti-cell migratory effect of SFN was associated with MMPs suppression of human oral squamous cell carcinoma (Jee et al. 2011).

Nowadays, combination therapy has become the hallmark of different types of cancer treatment due to the disease progression after monotherapeutic treatments. In this context, the SFN has combined effect with other medicinal agents to act synergistically against cancer (Kim et al. 2017; Bose et al. 2018; Aumeeruddy and Mahomoodally 2019; Mangla et al. 2019; Santana-Gálvez et al. 2020). A study by Kim et al. (2017) test the combination of SFN and paclitaxel and observed an increase in the activation of apoptotic signaling pathway members (caspase-3, caspase-8, and caspase-9 and cytochrome c) (Kim et al. 2017). In addition, the combined treatment downregulated the NF-κB signaling pathway, reducing the

protein expression of the apoptosis regulator genes of breast cancer. Bose et al. (2018) determined in a rats model, that SFN reduces DOX cardiotoxicity through Nrf2 activation while enhancing the killing of cancer cells by DOX (Bose et al. 2018). Another approach evaluated the effect of SFN, curcumin (C), and dihydrocaffeic acid (D, a chlorogenic acid metabolite) individually and in different combinations, over the viability of human colon cancer cells (HT-29 and Caco-2) (Santana-Gálvez et al. 2020). The best combination was SFN-D (1:1) since it was both synergistic and significantly more cytotoxic for colon cancer cells than healthy colon cells.

Several studies have shown that SFN exhibits anti-inflammatory activity by inhibiting NF-κB translocation and through the activation of Keap1/Nrf2 pathway, a mechanism that interrupts inflammatory signals to the nucleus (Vanduchova et al. 2019). Some approaches have presented SFN anti-arthritic and immunoregulatory activity (Table 15.1) (Kong et al. 2010; Davidson et al. 2013, 2017; Du et al. 2020). Kong et al. (2010) demonstrated that SFN inhibits synovial hyperplasia, activated T cell proliferation, and the production of IL-17 and TNF-α by rheumatoid arthritis (RA) T cells (Kong et al. 2010). Moreover, in a mouse model, SFN suppressed chronic autoimmune arthritis, inducing apoptosis in the proliferating synovium, at a high dose (200 μM). Another RA study revealed that activating Nrf2 by SFN profoundly inhibited the TNF-α-induced proliferation invasion, and MMPs expression in RA-fibroblast-like synoviocytes (RA-FLS) (Du et al. 2020). In pro-inflammatory cytokine-stimulated osteoarthritis (OA) study, SFN was able to suppress PGE2 or NO production from articular chondrocytes and inhibit proteoglycan and type II collagen degradation (Kim et al. 2012). The chondroprotective effect of SFN was also demonstrated by Davidson et al. (2013). SFN inhibits the expression of key MMPs implicated in OA, prevents inflammation at NF-κB pathway, and protects against cartilage destruction in vitro and in vivo (Davidson et al. 2013). Davidson et al. (2017) also conducted a human study to determine the detection of dietary isothiocyanates (ITCs) in knee joint (OA) patients and identify changes in the joint tissues. They demonstrate that a dietary bioactive with chondroprotective properties reaches the synovial fluid at concentrations with biological impact on the articular joint tissues (Davidson et al. 2017).

SFN has also attenuated other types of chronic inflammatory diseases (Table 15.1). Wu et al. (2019) demonstrated that SFN can reduce the level of inflammation in the skin of the atopic dermatitis (AD) mice model, reducing the accumulation of eosinophils and mast cells in the epithelial tissue (Wu et al. 2019). The effective target of SFN for the treatment of inflammatory skin diseases was also demonstrated by the downregulation of chemokines (TARC/CCL17 and MDC/CCL22) production in human keratinocytes (HaCaT) by inhibition of NF-κB activation (Jeong et al. 2010). Recently, the protective effects of SFN on brain health have been also demonstrated (Table 15.1) (Schepici et al. 2020). Hou et al. (2018) investigated the potential effects of SFN on amyloid-β (Aβ—a striking feature of Alzheimer's disease (AD) oligomer generation) (Hou et al. 2018). In vitro SFN improved cell viability and preserved dendritic length and in vivo SFN improved cognitive deficits, inhibited aggregation, and tau hyperphosphorylation,

as well as reduced the oxidative stress and neuroinflammation. SFN can also exert anti-inflammatory effects, reducing the neuronal damage mediated by microglial activation and reducing the synthesis of inflammatory mediators such as IL-1β, TNF-α, IL-6, and COX-2 (Klomparens and Ding 2019).

15.2.1.1 Sulforaphane and Their Therapeutic Associations: Trends on Nanomedicines for Cancer Treatment

Nanomedicine-based pharmacotherapy has been widely studied as innovative strategy for SFN delivery, especially for cancer treatment. In fact, its promising anticancer effects have driven efforts to overcome some limiting physicochemical properties such as chemical stability and low bioavailability (Tian et al. 2015; Wang and Bao 2021). In general, recent reports describe the development of new delivery systems, considering different routes of administrations and positions, but main innovations are related to their association with other drugs such as currently used anticancer therapies (docetaxel and cisplatin) and non-conventional drugs (acetylsalicylic acid, curcumin). This section will discuss the development and the main results obtained from those studies. Some of them are summarized in Table 15.2.

In the last years, several nanocarriers have been designed for SFN delivery, including polymeric, metallic, and lipid nanoparticles, micro and nanoemulsions, gels, and carbon dots. Among the most reported strategies authors propose the treatment of pancreatic cancer by oral route, which is considered an important factor to increase patient compliance. In this sense, Grandhi et al. (2013) reported the synthesis of solid lipid nanoparticles composed of stearic acid, as lipid phase, and poloxamer 188 as emulsion stabilizer, for encapsulating acetylsalicylic acid, curcumin, and SFN (Grandhi et al. 2013). The whole system chemopreventive effects were studied by *N*-nitrosobis-induced pancreatic cancer animal model, being effective at lower doses compared to other therapies, as well as reduced adverse reaction to the treatment. In another report, the same drug triad was used for avoiding pancreatic cancer progression by encapsulating them in a similar nanocarrier. However, chitosan was used as a stabilizer agent instead of poloxamer 188 to achieve best in vivo performances due to its positive charges, especially regarding bioadhesion to the small intestine and reduced uptake by the reticuloendothelial system (Thakkar et al. 2016). The use of non-steroidal anti-inflammatory drugs in association with SFN was also reported by the same authors. Ibuprofen was encapsulated into solid lipid nanoparticles with different lipid compositions, such as tripalmitin, stearic acid, and Compritol, stabilized by poloxamer 188 or tween 80. In this case, the ibuprofen-loaded nanoparticles and SFN coadministration showed synergistic effects by inhibiting the viability of human pancreatic cells (Thakkar et al. 2015).

In a more recent study, the association of curcumin and SFN was assessed by developing an ethosomal nanogel for skin cancer treatment. Although the study

Table 15.2 Summary of formulations sulforaphane (SFN)-loaded nanocarriers systems, their composition, and main results aiming cancer treatment

Nanomaterial	Composition	Main results	References
Carbon dots	SFN-conjugated carbon dots with thiourea groups	Enhanced targeting and imaging of epidermal growth factor receptor-overexpressing lung cancer cells	Lu et al. (2019)
Lipid nanoparticles	Nanostructured lipid carriers (Precirol®, ATO5, and Transcutol®)	Tamoxifen-SFN-coencapsulated nanoparticles showed increased intestinal permeability, oral bioavailability, and reduced in vivo toxicity	Mangla et al. (2020)
Metallic nanoparticles	Iron oxide–gold core-shell nanoparticles	Induction of apoptosis in human breast cancer cells (MCF-7) with decreased expression of Bcl-2 and Bcl-x_L	Manjili et al. (2016)
Metallic nanoparticles	PEGylated gold-coated iron oxide nanoparticles	SFN-curcumin co-loaded metallic nanoparticles evoked apoptosis in breast cancer cells	Danafar et al. (2017a)
Metallic nanoparticles	Gold nanoparticles	Enhanced cytotoxicity for B16-F10, MCF-7, SW-620, and Caco-2 cells and permeation across intestinal barrier	Soni and Kohli (2019)
Metallic nanoparticles	Tellurium flower-like nanoparticles	In vitro significant reduction of breast cancer cells viability and in vivo pancreatic accumulation	Krug et al. (2020)
Micelles	Monomethoxypoly (ethylene glycol)–poly(*ε*-caprolactone)	Enhanced cytotoxicity against human breast cancer cells (MCF-7)	Danafar et al. (2017b)
Micelles	Poly caprolactone–polyethylene glycol–poly caprolactone	Cytotoxic effects in MCF-7, 4T1 and MCF10A cells mediated by apoptotic events via BCL-2. SFN-loaded micelles evoked reduction in tumor dimensions and prolonged the drug mean residence time	Kheiri Manjili et al. (2017)
Nanocomposites	Silk fibroin in cerium-oxide-carbon dots	Theranostic strategy with enhanced efficacy and imaging in lung cancer cells	Passi et al. (2020)
Nanogel	Ethosomal gel	Enhanced efficacy against B16-F10 murine tumor cells for skin cancer treatment	Soni and Kohli (2019)
Peptide nanoparticles	Prolamin-based nanoparticles stabilized by sodium caseinate and propylene glycol alginate	SFN-encapsulated for colon-specific delivery showed controlled-release rate in simulated gastrointestinal fluid	Wang and Bao (2021)
Polymeric nanoparticles	Poly-lactide-*co*-glycolide-hyaluronic acid-nanoparticles	Docetaxel-SFN dual delivery was cytotoxic in docetaxel-resistant breast cancer stem cells and reduced β-catenin expression	Huang et al. (2016)

(continued)

Table 15.2 (continued)

Nanomaterial	Composition	Main results	References
Polymeric nanoparticles	Poly-L-glutamic acid–cisplatin conjugates	Cisplatin-SFN-nanoparticles showed enhanced cell internalization, tumoral accumulation, and antitumor effects	Xu et al. (2019)
Solid lipid nanoparticles	Stearic acid stabilized by poloxamer 188	Association of acetylsalicylic acid, curcumin, and SFN-encapsulated in solid lipid nanoparticles with synergistic antitumor activity in pancreatic cancer animal model	Grandhi et al. (2013)
Solid lipid nanoparticles	Stearic acid, Compritol 888 ATO, or tripalmitin stabilized by poloxamer 188, tween-80	SFN-ibuprofen-loaded solid lipid nanoparticles showed synergistic cytotoxic effects in in vitro pancreatic cancer cells	Thakkar et al. (2015)
Solid lipid nanoparticles	Stearic acid stabilized by chitosan	Acetylsalicylic acid, curcumin, and SFN-encapsulated with low toxicological profile and enhanced intestinal bioadhesive properties for pancreatic cancer treatment	Thakkar et al. (2016)

reports mainly physicochemical aspects, promising antitumor effects were achieved after B16-F10 cell treatment (Soni et al. 2020).

In other reports, the association of SFN with conventional anticancer therapy has also shown promising results. For example, SFN-docetaxel co-loaded PLGA-hyaluronic acid polymeric nanoparticles were studied for avoiding the initiation and progression of breast cancer, including possible metastasis episodes. In this in vitro study, breast cancer stem cells with recognized docetaxel-resistant phenotype were treated with both drugs docetaxel and SFN, where SFN-loaded nanoparticles induced more pronounced cytotoxic effects than that compared to non-encapsulated drugs and, additionally, reduced the expression of β-catenin. In a complementary way, in vivo tests revealed an enhanced antitumor efficacy by SFN and docetaxel-loaded nanoparticles (Huang et al. 2016).

The synergistic effects of SFN with tamoxifen were also investigated for breast cancer therapy. In an attempt to avoid the extensive tamoxifen first-pass metabolism, Mangla et al. (2019) developed nanostructured lipid carriers with different stabilizers (poloxamer 188 or tween 80) for promoting tamoxifen permeation across the intestinal barrier and, simultaneously, inhibit P-glycoprotein efflux transporter activity (Mangla et al. 2019). Those strategies improved the tamoxifen uptake by cancer cells and increased their sensitivity to SFN, explaining the synergism between both therapeutic agents. Subsequently, another study with a similar strategy also reported a possible optimization of dosing and administration frequency, associated with reduced tamoxifen toxicity, when compared to non-encapsulated drugs (Mangla et al. 2020).

Innovative alternatives to overcome conventional drug limitations were also emphasized by other authors (Xu et al. 2019). The cisplatin chemosensitivity restoration was their main purpose when synthesizing poly-L-glutamic acid–cisplatin conjugates associated with SFN. The increased nanoparticles' cellular internalization was able to modulate the glutathione depletion, which promoted the cisplatin capability for DNA binding, resulting in enhanced cell death effects by apoptosis in breast cancer cells.

In addition to therapeutic associations, new SFN-loaded nanocarriers have been reported, especially considering the development of hybrid systems with multifunctional properties. One of the main strategies refers to the design of metallic nanoparticles, for example, gold-coated iron oxide nanoparticles functionalized with thiolated-polyethylene glycol–folic acid, as reported by Manjili et al. (2016). Physicochemical characterization techniques revealed the synthesis of a stable system able to induce apoptosis mechanisms in MCF-7 human breast cells cancer, such as decreased expression rate of anti-apoptotic genes (Bcl-2 and Bcl-x_L). In a similar study, other authors reported the considerable cytotoxic effects of SFN-loaded tellurium flower-like nanoparticles in two breast cancer cells lines (MCF-7 and MDA-MB-231) when compared to normal cells (MCF-10A) (Krug et al. 2020).

Another recent innovation is the use of versatile nanocarrier systems applied to theranostic purposes. Passi et al. (2020) described multifunctional materials based on SFN-loaded silk fibroin and their further association with cationic cerium oxide nanoparticles and carbon dots (Passi et al. 2020). In fact, the whole system multiple functions are resulting from the association among green fluorescence emission, antioxidant and anticancer activity attributed to carbon dots, cerium oxide nanoparticles, and SFN-loaded silk fibroin, respectively. This multifunctional nanocomposite efficiently reduced the reactive oxygen species levels and allowed better resolution fluorescence images from both tumoral (A549) and normal (L132) lung cells. In a similar report, SFN-carbon dots conjugates functionalized with thiourea groups were developed for targeting and imaging epidermal growth factor receptor-overexpressing lung cancer cells (Lu et al. 2019).

15.3 Conclusion

Nanomedicines have been described as one of the most promising alternatives for overcoming physicochemical and biopharmaceutical limitations of a variety of drugs. These advantages are especially useful for improving the pharmacological effects of conventional therapeutics. On the other hand, phytochemicals, such as SFN, have been proposed as new pharmacotherapy, which expands the research for the treatment of some diseases such as chronic inflammatory processes and cancer. Since polytherapy is the gold-standard treatment, dose adjustments, changes in routes of administration, and possible side effects are factors that must be considered. In this sense, several nanocarriers (micelles, organic and inorganic nanoparticles, nanocomposites, etc.) exert an essential role for developing more

effective and safe formulations. In the case of SFN, its incorporation into nanosystems evoked an improvement in cytotoxic and anti-inflammatory effects, with special attention to elucidating the molecular mechanisms involved.

Funding This research was funded by The São Paulo Research Foundation, Grant numbers 2019/20303-4 (D.R.A.), Brazilian National Council for Scientific and Technological Development, Grant number 307718/2019-0 (D.R.A.) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil, Grant number CNPq #308819/2022-5 (CAPES, finance code #001).

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