REVIEW

Acrolein scavengers: Reactivity, mechanism and impact on health

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Acrolein (ACR) is an α,β-unsaturated aldehyde that exists extensively in the environment and (thermally processed) foods. It can also be generated through endogenous metabolism. Its high electrophilicity makes this aldehyde notorious for its facile reaction with biological nucleophiles, leading to the modification of proteins/DNA and depletion of glutathione. Recent studies also have revealed its roles in disturbing various cell signing pathways in biological systems. With growing evidences of ACR's implication in human diseases, strategies to eliminate its hazardous impacts are of great importance. One of the intervention strategies is the application of reactive scavengers to directly trap ACR. Some known ACR scavengers include sulfur (thiol)-containing and nitrogen (amino)-containing compounds as well as the newly emerging natural polyphenols. In this review, the interactions between ACR and its scavengers are highlighted. The discussion about ACR scavengers is mainly focused on their chemical reactivity, trapping mechanisms as well as their roles extended to biological relevance. In addition to their direct trapping effect on ACR, these scavengers might possess multiple functions and offer additional benefits against ACR-induced toxicity. A comprehensive understanding of the mechanism involved may help to establish ACR scavenging as a novel therapeutic intervention against human diseases that are associated with ACR and/or oxidative stress.

Keywords:
Acrolein / Antioxidants / Lipid peroxidation / Scavengers

1 Introduction

2-Propenal, known as acrolein (ACR), is a highly electrophilic α,β-unsaturated aldehyde that exists as colorless to yellowish flammable liquid with an irritating odor at room temperature. It is used as an indispensible intermediate in the synthesis of many organic chemicals/materials for industrial-scale production [1]. In addition to its industrial release, its environmental presence is principally attributed to the incomplete combustion of a wide range of organic matters including petrol, coal, plastics, wood materials and tobacco [2]. ACR is also found in the foodstuffs (cocoa beans, chocolate liquors, fried potatoes, etc.) or from the volatiles generated during the thermal treatment of animal or vegetable fats at high temperatures [3, 4]. Moreover, ACR is regarded as an endogenous product from myeloperoxidase-mediated degradation of threonine, amine oxidase-mediated degradation of spermine and spermidine [5], metabolism of a group of widely used alkylating oxazaphosphorines (anticancer drugs) [6] and free radical-initiated lipid peroxidation of polyunsaturated fatty acids in cell membranes [7–10].

The presence of two reactive moieties in ACR: C=C double bond and C=O carbonyl group in a conjugated

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Abbreviations: ACR, acrolein; **AGEs,** advanced glycation end products; **D3T,** 2-dithiole-3-thione; **dG,** deoxyguanosine; **DIMESNA,** dithio-bis-mercaptoethanesulphonate; **DTP,** 6-dithiopurine; **EGCG,** epigallocatechin-3-gallate; **FDP-Lys,** *N*ε-(3-formyl-3,4-dehydropiperidino) lysine; **GO,** glyoxal; **GSH,** glutathione; **GST,** GSH transferases; **HNE,** 4-hydroxynonenal; **LA,** lipoic acid; **MDA,** malondialdehyde; **MESNA,** 2-mercaptoethanesulfonate; **MGO,** methylglyoxal; **NAC,** *N*-acetylcysteine; **NF-κB,** nuclear factor-κB; **RCS,** reactive carbonyl species; **RNase A,** ribonuclease A; **ROS,** reactive oxygen species

C═C–C═O system, is responsible for its pronounced electrophilicity. ACR is suggested as the strongest electrophile among all α,β-unsaturated aldehydes; therefore, it has the highest reactivity towards biological nucleophiles, which leads to its strong toxicity [11]. Due to its solubility in water, alcohol and diethyl ether, ACR can easily travel across cell membranes by passive diffusion [12]. In the past, extensive studies have been conducted to establish the toxicological profile of ACR in cells, tissues and animals. In general, ACR exerts its toxic effects mainly through disrupting the function of proteins/DNA by direct addition, lowering intracellular glutathione (GSH) levels, and interfering with cell signaling pathways as discussed in the following sections.

For proteins, the nucleophilic sites such as sulfhydryl group of cysteine, ε-amino group of lysine and imidazole moiety of histidine are the main targets of ACR. Among these sites, the preferential formation of Michael-type adducts on cysteine residues is believed to be the predominate way for ACR to exert its reactivity in biological systems [13]. Amino groups present in cellular molecules such as lysine-containing proteins are also targets for the attack by ACR. As an example, *N*ε-(3-formyl-3,4-dehydropiperidino) lysine adduct (FDP-Lys) has been identified as an ACR-lysine adduct in human low-density lipoproteins (LDL) [8]. The subsequent development of antibodies for the specific detection of FDP-Lys becomes an important method for the measurement of protein-bound ACR, which has wide applications in studies focusing on oxidative stress [9, 14]. Furthermore, the inter- and intra-molecular protein cross-links can also occur as the initial addition of ACR to proteins can give rise to adducts containing electrophilic centers that can participate in additional deleterious reactions [15].

Similar to proteins, the deoxyguanosine (dG) residues in DNA serve as nucleophiles to form cyclic 1, *N*-propano-deoxyguanosine adducts (α-hydroxy-PdG and γ-hydroxy-PdG) with ACR [16]. Interstrand DNA cross-links can also be formed between guanines in the neighboring CG and GC base pairs located in 5′-CpG-3′ sequences, resulting from the opening of the 8-hydroxypropano ring to the corresponding aldehyde [17]. The DNA modification by ACR is anticipated to disturb the replication and transcription of DNA, thereby contributing to the etiology of diseases. As a result, ACR-derived guanosine adducts have been detected as a prevalent lesion in human and rodent tissues with potential significance in carcinogenesis [18, 19].

GSH is a critical endogenous antioxidant involved in cell defense [20]. The conjugation of ACR to GSH is regarded as a major pathway for the detoxification of ACR [21]. ACR may lead to the depletion of cellular GSH in a concentration-dependent manner, which may cause harmful effects to human beings. It is documented that once the GSH level has reached a certain threshold, protein thiol groups could be progressively modified while a series of molecular effects may emerge such as cell proliferation, apoptosis and changes in gene/protein expression [22, 23]. Detailed interaction between ACR and GSH will be discussed in the part of sulfur-containing scavengers in this review.

The electrophilic nature of ACR has been extensively discussed in numerous literature reports. However, only in recent years, more and more attention has been paid to the subtle molecular mechanisms responsible for ACR's harmful effects. ACR has been reported as a modulator of several transcription factors such as nuclear factor B (NF-κB) related to stress-sensing, activator protein 1 (AP-1) related to a variety of stimuli and nuclear erythroid-2 related factor 2 (Nrf2) related to cellular redox status [12, 22]. It is also suggested that the disturbing action of ACR in various cell signaling pathways may further help to diminish cell proliferation and probably increase the susceptibility of cells to death via apoptosis and oncotic necrosis.

As a secondary product generated in lipid peroxidation, ACR is often studied together with other products formed in lipid peroxidation such as 4-hydroxynonenal (HNE), malondialdehyde (MDA) and glyoxal (GO). All these compounds are termed as 'reactive carbonyl species (RCS)', which can be formed as the consequence of oxidative processes initialized by reactive oxygen species (ROS). Accumulating evidences suggest that RCS, rather than ROS, may mediate the progression of some oxidative-related chronic diseases. Upon the successful detection of FDP-lysine (one of the indictors of oxidative stress) using Western blotting in human tissues, ACR has been found to be closely associated with a number of diseases including Alzheimer's disease, atherosclerosis, diabetes, traumatic spinal cord injury and cigarette smoke-induced lung cancer [14, 24–28]. Pharmacological efforts to attenuate the toxicity of ACR have received much attention in recent years. According to many studies, the defense against oxidative stress is believed to be a major event involved in the inhibition of ACR's harmful effects. Meanwhile, the direct trapping of ACR by chemical agents may be another important way contributing to the amelioration of ACR's toxicity. Therefore, compounds possessing both antioxidant activity and direct scavenging capacity for ACR should receive much attention, e.g. some sulfur (thiol)-containing compounds, nitrogen (amino)-containing compounds, naturally occurring polyphenols as well as ascorbic acid.

2 Sulfur (thiol)-containing compounds as ACR scavengers

2.1 Reactivity

The facile reactivity of thiols with unsaturated aldehydes makes them good candidates for detoxification of ACR. It has been demonstrated that cysteine residues were the targets for glycation and low molecular mass thiols may act as protective agents against dicarbonyl-induced toxicity [29]. In the case of ACR, the presence of an unsaturated carbonyl group allows it the capacity to form stable covalent adducts with nucleophilic thiols by Michael-type addition [30]. The

structures of some sulfur (thiol)-containing compounds commonly referred as ACR scavengers are shown in Fig. 1.

The rate and equilibrium constants for ACR to react with cysteine and GSH were firstly established by Esterbauer et al. [31, 32]. In 0.066 M phosphate-buffered saline (PBS, pH 7.4) at $20 \pm 1^{\circ}C$, the rate constant k_1 for the forward reaction to form ACR-thiol adducts was about 220 and 121 $M^{-1} s^{-1}$ for cysteine and GSH, respectively, while the k_2 for reverse reaction was only 5.0×10^{-6} and 1.76×10^{-6}, respectively, indicating the thiol adducts of ACR are considerably stable. Under slightly different condition (0.1 M PBS, pH 7.3, with initial concentration of ACR ten times higher than that of thiol compounds), the equilibrium constant K (mol^{-1}) was found to be 5.4 and 0.53 for cysteine and GSH, respectively [33]. The higher reactivity towards thiol group of cysteine than that of GSH was explained by the close vicinity of the amino groups in the cysteine and its possible cyclization to form a thiazolidine derivative with the equilibrium shifting to the right [33]. In addition, ACR reacts about 110–150 times faster with GSH than crotonaldehyde and HNE on account of the lack of any electron-releasing substituent on its C-3 position (the substitution lessens the partial positive charge and leads to the decrease of the electrophilic reactivity of the double bond) [31].

In addition to cysteine and GSH, the reactivities of other sulfur (thiol)-containing compounds have also been studied.

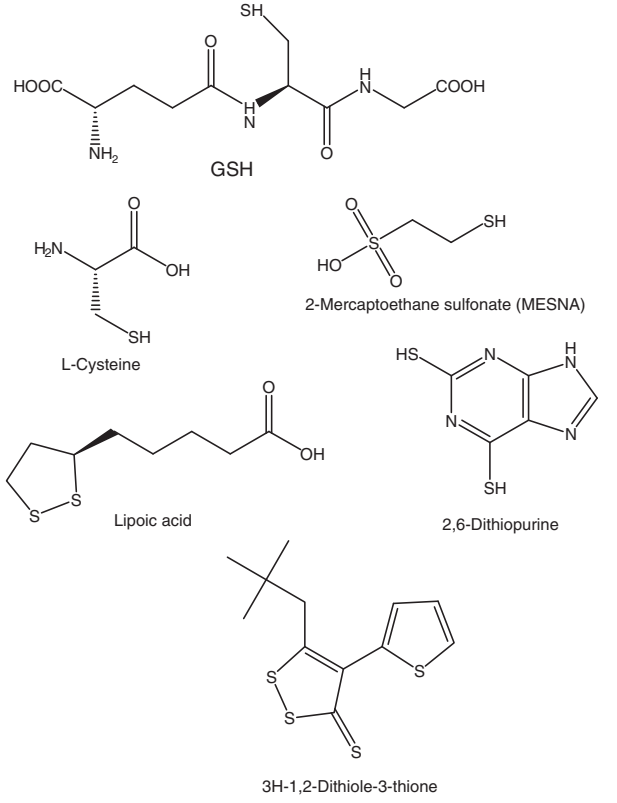

Figure 1. The chemical structures of several sulfur (thiol)-containing compounds as acrolein scavengers.

2-mercaptoethanesulfonate (MESNA) is a small molecule which was developed for trapping of ACR by its thiol group. With the technique of headspace solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC-MS), ACR was quantified and MESNA (10 μM to 20 mM) was demonstrated to be a potent quencher of ACR (1–10 000 nM), dose-dependently in both PBS and human urine. It is also pointed out that MESNA works more effectively under alkaline conditions [34]. In addition, it was reported that a complete inactivation of ACR (0.5 mM) was achieved by MESNA (0.5 mM) within 10 min in buffered solutions at neutral pH and 37°C [35]. Lipoic acid (LA) is a naturally occurring precursor of an essential cofactor for mitochondrial enzymes with two vicinal sulfur atoms linked via a disulfide bond. With the assistance of MS, the 1:1 adducts of reduced LA and ACR were observed with 30 min incubation at 25°C in 5 mM KH_2PO_4 buffer (pH 7.2) [36]. 2,6-Dithiopurine (DTP) belongs to the family of potential cancer chemopreventive agents, purinethiols with nucleophilic scavenging activity. When ACR (13.5 mM) was incubated with DTP (47.5 μM) in 50 mM PBS (pH 7.5) at 37°C, there was a decrease in the UV absorbance of DTP at 285 nm and the rate constant was determined to be 1.10 $M^{-1} s^{-1}$ [37].

2.2 Mechanism

It was reported that the initial reaction between ACR and GSH takes place at C-3 position of ACR with the GSH residue through binding by a thio-ether linkage, and this reaction is generally assumed to be the rate-determining step. This facile reaction consists of nucleophilic attack at C-3, leading to the formation of a 1,4 Michael addition product that is further tautomerized to the aldehyde with a 1,2 addition across the olefinic double bond [13] (Fig. 2A). As shown in Fig. 2A, there is no cyclization in the 1:1 adducts of ACR-GSH and the free aldehyde group is not involved in further reactions with GSH [21]. Different for GSH, when cysteine is incubated with ACR, the aldehyde group of ACR is another target for the thiols to react with. The addition of another cysteine molecule to the mono-adduct is very fast with the formation of the 1:2 aldehyde-thiol adduct with a thiazolidine ring formed through intramolecular cyclization (Fig. 2B). It has also been pointed out that the free aldehyde group in ACR is also a target for the Schiff base with amino groups. This type of reaction is less facile than Michael addition but can be subsequently accelerated with the initial formation of Michael addition reaction [21, 38].

2.3 Impact on health

Cellular sulfur (thiol)-containing compounds are regarded as important ACR detoxification agents through the facile

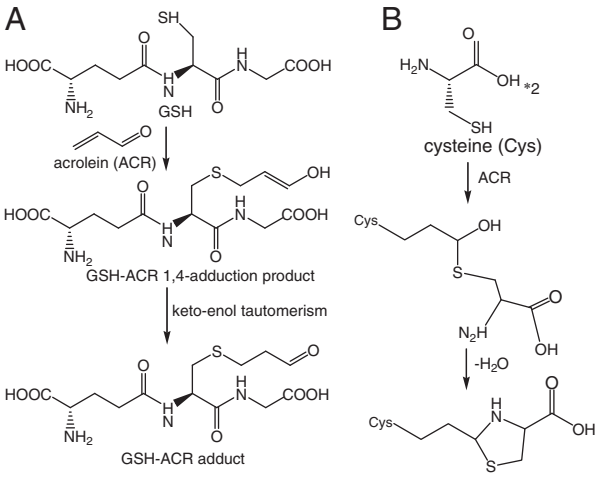

Figure 2. The reaction mechanism of representative sulfur (thiol)-containing compounds GSH (A) and cysteine (B) in scavenging of acrolein.

formation of thiol-ether adducts. GSH is the most abundant non-protein thiol with millimolar concentration in most parts of the cells such as in the cytosol (1–11 mM), nuclei (3–15 mM) and mitochondria (5–11 mM) [39]. It is able to counter oxidative stress and can directly scavenge hydroxyl radical and singlet oxygen, thus detoxifying hydrogen peroxide and lipid peroxides with the assistance of GSH peroxidase [40]. Furthermore, it can effectively scavenge RCS including ACR. Exposure of cells to ACR was reported to lead to a dose-dependent loss of cellular GSH upon conjugation and the formation of conjugated ACR-GSH adducts [23]. The conjugated ACR-GSH is then further metabolized by mitochondrial and cytosolic aldehyde and alcohol dehydrogenase to form novel metabolites. One of the major metabolites has been identified as 3-hydroxypropylmercapturic acid (3-HPMA) in laboratory animals [41, 42] and in urine of cigarette smokers [43]. However, the reports of application of exogenous GSH as an approach to counteract ACR are scarce. It was reported that when ACR was incubated with purified cytochrome P450 in the absence of NADPH, 90% of binding of [^{14}C] ACR to hepatic microsomes was inhibited by GSH [44]. It was also reported that the depletion of GSH caused hypersensitivity of *Escherichia coli* to ACR, and the resistance conferred by GSH was assumed to be largely due to its direct interaction with ACR [45]. The fluctuation of GSH level and its ACR-scavenging rate can also be attributed to other indirect causations such as the inhibition of enzyme activity related to GSH's detoxification activity (e.g. GSH transferases (GST), which are known to mediate the formation of ACR-GSH adducts). For instance, the concomitant treatment with sulfasalazine (an established inhibitor of GST) markedly amplified the ACR-induced cytotoxicity in cultured neonatal vascular smooth muscle cells [46], while the enhanced resistance to ACR was achieved in GST A4-4 transfected mouse pancreatic islet endothelial cells with significantly higher GSTs enzyme activity [47].

Therapeutic strategies to restore or increase intracellular levels of GSH have been regarded as an important way to detoxify ACR. However, dietary GSH supplementation is considered less effective due to the hydrolysis of GSH by intestinal and hepatic γ-glutamyltransferase. It was reported that the circulating GSH cannot be increased to a clinically beneficial extent by oral administration even with a high dose of 3 g of GSH [48]. Alternatively, the augmentation of GSH level by its precursors for synthesis such as *N*-acetylcysteine (NAC) has been well documented [49–51]. NAC is an amine-protected version of cysteine which can be rapidly hydrolyzed back to cysteine in human body [52]. It is commercially available as a dietary supplement commonly claimed as an antioxidant and liver protecting agent. The exogenous administration of NAC or cysteine has received attention to attenuate ACR-related toxicity [38]. As indicated in protein models, the inhibited activities of human PON-1 (aryldialkylphosphatase, EC 3.1.8.1) [53] and human antithrombin (AT) by ACR were attenuated with the addition of cysteine [54]. In the cell models, the protective effect of NAC against ACR was observed, as evidenced by the increased cell survival [55–57], as well as in the regulation of multiple factors related to cell viability. For instance, NAC is associated with a number of beneficial cellular events such as the reversal of DNA damage in human lymphoid cells (Raji) [58]; the reduction of carbonylation level in cigarette smoke extract-treated human bronchial epithelial cell line BEAS-2B [59]; the decreased interleukin-8 (IL-8) release associated with damage to the lung epithelium of human lung fibroblasts cells [60]; the restrained production of matrix metalloproteinases (MMPs) that is related to the degradation of the extracellular matrix [61]; and the reduced oxidation of thioredoxin (Trx1) in human microvascular endothelial cells which is critical in the maintenance of cellular thiol redox balance [62]. In these studies, the maintenance of GSH level is regarded as a major justification for the resistance towards ACR, although the direct trapping mechanism has not been clearly presented. Similar results are also found in some in vivo studies. One study with rodents showed that intraperitoneal injection of NAC significantly reduced synaptosomes carbonylation caused by the exposure to ACR. The levels of protein-conjugated ACR at the locus of infarction and in plasma were also remarkably reduced by the administration of NAC after the induction of infarction by 0, 3 and 6 h. Controversially, in a rat model of inhalation injury, single doses of NAC (1 g/kg) given intravenously 15 min prior to the exposure to ACR vapor (210 ppm) showed no significant improvements with either mortality or pulmonary histological damage [63].

MESNA is another thiol-containing nucleophile and an adjuvant designed specifically to quench ACR for patients receiving the alkylating oxazaphosphorine-type cancer chemotherapeutic agents. As a metabolite of cyclophosphamide and ifosphamide, ACR might be responsible for

mucosal irritation that leads to hemorrhagic cystitis. Through direct trapping, MESNA can block ACR from getting into the uroepithelium in clinical observations [64]. MESNA was found to be potent in reversing the suppression of antibody-forming cell (AFC) response induced by ACR in splenocytes of B6C3Fl mice [65], lessening cell injury reflected by the improvement of neutral red dye uptake, glucose transport and GSH content in the primary culture of rabbit proximal renal tubule cells [66], and completely blocking the stimulated expression of IL-8 in cultured normal human lung fibroblasts and small airway epithelial cells [60]. Compared with GSH, MESNA (1 mM) was found to be more effective to block ACR-induced damages on chondrogenesis in mouse limb bud mesenchymal cells [67]. Evidences from animal studies confirmed that MESNA (injected intravenously or intraperitoneally) reduced the vascular permeability and bladder weight increase caused by ACR [68]. In the human body, MESNA can undergo rapid oxidation in the plasma to form a disulfide, dithio-bis-mercaptoethanesulfonate (DIMESNA) [69]. MESNA and DIMESNA have been investigated in renal tubular cell line LLC-PK1 for their effects on ACR-induced toxicity. The treatment of MESNA reversed the trend of severe loss of total proteins and the incorporation rates of thymidine/uridine caused by ACR. However, DIMESNA failed under the same experimental conditions. It is reasonable to presume that only MESNA functions as an ACR quencher [70].

LA is also considered as an organosulfur-type ACR scavenger. It has one chiral center and exists in two enantiomers as R-(+) and S-(−)-LA. The R-form of LA is the only enantiomer existing in nature and is an essential cofactor of mitochondrial bioenergetic enzyme complexes [71]. LA has been well studied as a potent antioxidant for the detoxification of heavy metals and for the improvement of age-associated cognitive decline [71, 72]. The pretreatment of human pigment epithelial ARPE-19 cells with R-LA could abolish the cytotoxicity induced by both acute (24 h) and continuous exposure (up to 8 days) to ACR. In human neuroblastoma SH-SY5Y cells, the levels of GSH, NAD(P)H:quinone oxidoreductase 1 (NQQ1), and mRNA expression of γ-glutamylcysteine ligase catalytic subunit (GCLC) were all up-regulated by LA, which might be relevant to cell survival against acrolein-induced toxicity [73]. The protective effect of R-LA against ACR-induced toxicity was also observed in human lung fibroblast cell IMR-90, through regulation of the mitochondrial membrane potential, superoxidase dismutase activity and levels of GSH, ATP and ROS [74]. The abundant supplement from dietary sources and the wide distribution in cellular membranes/cytosol [71] make LA a promising candidate in detoxification of ACR. Additionally, the supplement of LA could enhance the content of cysteine and GSH which is related to age-associated decline in thiol redox ratio [75]. This might be another explanation for its potency against ACR-induced toxicity. Investigations aiming at alleviating the toxicity of ACR were further extended to derivatives of lipoid acid. Dihydrolipoic acid (DHLA) was recently discovered as an inhibitor against the formation of ACR-derived 1,*N*(2)-propanodeoxyguanosine (Acr-dG), which was detected in the reaction of ACR with dG as well as in the reaction of docosahexaenoic acid (DHA) with dG in the presence of $FeSO_4$ [76]. Lipoamide (LM), the neutral amide of LA, was also reported to be more effective than LA to protect human retinal pigment epithelial (RPE) cells against the formation of ACR-induced oxidant formation, mitochondrial dysfunction, DNA damage and increased protein carbonyl level [77].

Several additional organosulfur compounds including DTP, 3H-1,2-dithiole-3-thione (D3T) and sulforaphane have also been investigated for their health benefits. DTP was originally introduced as a reactive nucleophile that can quench electrophilic carcinogens. When administrated together with cyclophosphamide, DTP was demonstrated to prevent the lung and bladder damage induced by cyclophosphamide in mice, although the ACR-DTP adduct was not detected in the mice urine by mass spectrometric analysis [78]. D3T is a prototype of dithiolthiones. It presents abundantly in cruciferous vegetables and regarded as a potent inducer of enzymes/factors for fending off electrophile carcinogens [79]. In human SH-SY5Y neuroblastoma cells and rat aortic smooth muscle A10 cells, the improved cell viability and morphological changes were observed with the treatment of cells with D3T under ACR challenge [80, 81]. This was reported to be achieved through the regulation of γ-glutamylcysteine synthetase (γ-GCS) and GST activity with increased contents of GSH [81]. Another organosulfur compound sulforaphane derived from cruciferous vegetables could also attenuate ACR's cytotoxity in A10 cells, rat liver RL-34 epithelial cells and mouse embryonic fibroblasts possibly through a similar mechanism [82, 83].

3 Nitrogen-containing compounds as ACR scavengers

3.1 Reactivity

Compared with sulfur-containing scavengers, nitrogen-containing compounds (Fig. 3) might be less powerful with respect to the kinetics of carbonyl-sequestering reactions. However, some recent studies, which aimed at identifying new classes of carbonyl scavengers with acceptable pharmacokinetic properties, have identified some of these compounds as downstream mediators against oxidation-introduced carbonyl stress.

One of the examples is hydralazine which was demonstrated to possess strong ACR-trapping reactivity. It was reported that in a cell-free system, the content of ACR (0.5 mM) was diminished to nearly 8% of the initial concentration after 30 min incubation with hydralazine

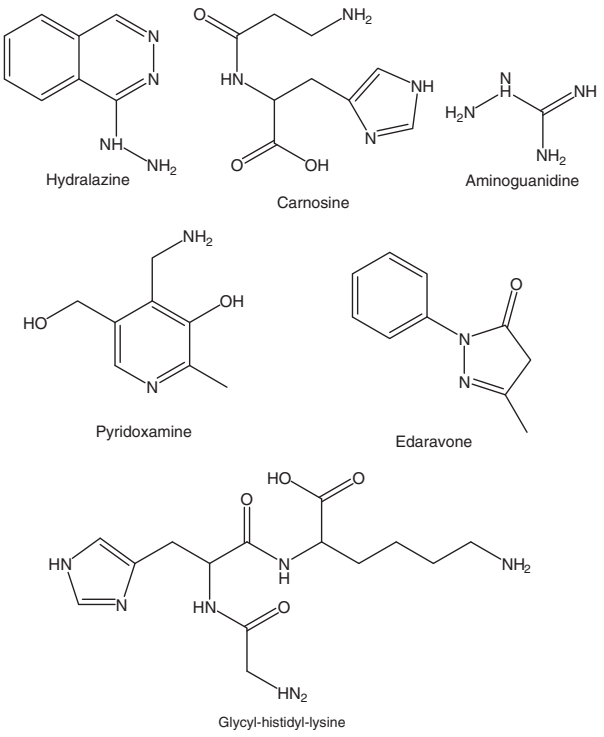

Figure 3. The chemical structures of several nitrogen (amino)-containing compounds as acrolein scavengers.

(0.5 mM) in PBS (pH 7.0). Under the same condition, dihydralazine was found to show even stronger ACR-scavenging capability with only 1% of ACR remaining [35]. With the availability of large amount of hydrazinoaromatic compounds, the study of structure–activity relationship has also been carried out and some conclusions were made as follows: to trap ACR in buffered solution at neutral pH and 37°C, the hydralazine analogues with two ring nitrogen atoms can facilitate the reactivity. The reduced number of ring nitrogen atoms leads to the decrease of activity, which makes naphthylhydrazine (lacking any ring heteroatom in its rings) as a least active scavenger. Finally, it has been pointed out that comparing with other nitrogen-containing nucleophiles, hydrazine-type compounds exhibit a higher reactivity, but they are still less effective than the thiol-containing chemicals such as MESNA [11, 84].

Carnosine and its analogues are known as a group of aminoacyl histidine dipeptide-type quenchers of ACR. It was reported that a 3-h incubation of 30 μM of ACR with 1 mM of chemical agents including carnosine, homocarnosine, β-alanine, anserine, γ-aminobutyric acid, *N*-acetylcarnosine and *N*-acetylhistidine in 1 mM PBS (pH 7.4) at 37°C significantly reduced the amount of ACR, with carnosine, homocarnosine and anserine being the most effective ones. It was suggested that the constituent amino acids in carnosine (β-alanine, γ-aminobutyric acid, *N*-acetylhistidine) may act cooperatively and both the terminal amino group and the nitrogen atoms of the imidazole ring of the L-histidyl residue are involved in the trapping reaction [85]. Compared with carnosine and their analogues, aminoguanidine and pyrodoxamine are less reactive towards ACR. Aminoguanidine displayed little or no ACR-trapping potency when it was incubated together with ACR at 37°C and neutral pH [84, 86]. In the case of pyridoxamine, it was found that 25.5% of ACR remained in PBS (pH 7.4) after 3 h incubation with pyridoxamine at 37°C [87]. Edaravone is a newly developed radical scavenger that has been approved as a neuroprotective agent by the Japanese health authorities. It was reported that after edaravone (1 mM) was incubated with ACR (50 μM) at 37°C for 3 h in PBS (10 mM, pH 7.4), the amount of ACR was dramatically reduced to 0.4%. Such a scavenging activity was much stronger than the selected reference compounds (pyridoxamine, carnosine and hydralazine) under the same reaction condition [87]. An endogenous tripeptide, glycyl-histidyl-lysine (GHK) was also discovered to possess ACR-sequestering capability in a time- and dose-dependent manner. It was reported that after incubation of the GHK peptide (1 mM) with ACR (30 μM) for 4 h in PBS (1 mM, pH 7.4) at 37°C, a complete quench of ACR was achieved [88].

3.2 Mechanism

The amino groups in nitrogen-containing ACR scavengers generally are the nucleophilic targets for α,β-unsaturated aldehydes, with the nucleophilic attack occurring through the formation of Schiff base products or Michael addition products.

As a hydrazine derivative, hydralazine may trap ACR as does 2,4-dinitrophenylhydrazine (DNPH), a well-known derivatization reagent for the analysis of carbonyl compounds. The trapping reaction proceeds by the nucleophilic addition to ACR followed by 1,2 elimination of one molecule of water to form hydrazone. The products formed from the reaction between hydralazine and ACR were identified as (1E)-acrylaldehyde phthalazin-1-yl-hydrazone (E-APH) with 35% yield and (1Z)-acrylaldehyde phthalazin-1-ylhydrazone (Z-APH) with 4% yield [35] (Fig. 4A).

The trapping of ACR by carnosine is reported to form a cascade of intermediates and final products, of which only some are described in Fig. 4. Compound 1 was reported to be generated from intramolecular Michael addition of ACR to the histidyl residue. It could further undergo an imine bond formation with amino-terminal group of β-alanine in carnosine to give rise to the predominant compound, compound 2 with a 14-membered macrocyclic structure. The Michael addition reaction could also proceed with the amino group of β-alanine and two molecules of ACR. Eventually, 3*N*β-(3-formyl-3,4-dehydropiperidino) derivatives can be produced through aldol condensation and dehydration [85].

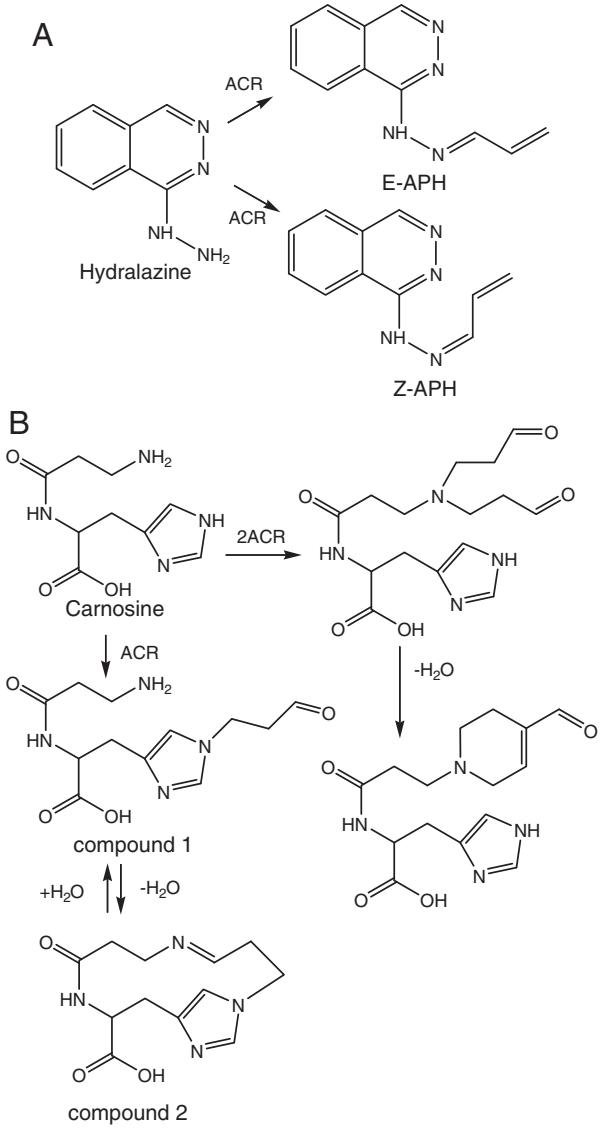

Figure 4. The reaction mechanism of representative nitrogen (amino)-containing compounds hydralazine (A) and carnosine (B) in scavenging of acrolein.

3.3 Impact on health

Hydralazine has been known as an antihypertensive agent for over 50 years. It was reported to exert its vasodilatory action through direct relaxation of the vascular smooth muscle cells [89]. Some strong nucleophilic groups in its structure also render its possible scavenging capability of several biogenic ketones and aldehydes under certain physiological conditions [90]. In a model of ACR-modified BSA at neutral pH and 37°C, hydralazine was found to attenuate the carbonylation level with better effects than other scavengers such as aminoguanidine and carnosine. In the same study, hydralazine was also found to protect isolated mouse hepatocytes from cell death, which was mediated by ACR derived from in situ alcohol dehydrogenase-catalyzed metabolism of allyl alcohol [84]. In another cell-free model system, the formation of cross-linked bovine pancreas ribonuclease A (RNase A) dimer and trimer induced by ACR was found to be inhibited by hydralazine, consistent with lower amount of ACR-modified lysine residues and protein carbonyl level [91]. In cell model studies, positive roles of hydralazine have also been observed. In rat adrenal pheochromocytoma cells, hydralazine was found to significantly reduce the ACR-caused membrane damage, disruption of mitochondrial functions and intracellular GSH depletion, thus leading to a higher cell survival rate [92]. Further supporting evidence comes from the exposure of rat heart homogenate to allylamine (a compound that could be converted into ACR by amino oxidase). After the addition of allylamine for 6 h, the supplementation of hydralazine and its analogues phenelzine and procarbazine was found to significantly inhibit the production of ACR [93]. In another in vitro model of spinal cord injury, hydralazine was also found to significantly reduce the formation of ACR-Lys adducts, which is regarded as one of the potential mediators of secondary injury [94]. Consistent with the above studies, the in vivo hepatoprotection offered by hydralazine under allyl alcohol stress was found to be accompanied by concentration-dependent ACR-trapping effects in total liver proteins ranging from 26 to 200 kDa in mice. In-depth immunohistochemical analysis of liver slices revealed the distribution of diffuse, extranuclear adduct-trapping (reaction between hydralazine and protein-adducted ACR) was in liver lobule and in parenchymal cell membranes [95]. In addition, a significant elevation of ACR-protein adduct levels was also observed in the spinal cord of experimental autoimmune encephalomyelitis mice (the model of multiple sclerosis). The medication with hydralazine (received by daily intraperitoneal injections) was reported to significantly improve behavioral outcomes and lessen myelin damage in spinal cord [96]. Finally, in both rabbit femoral artery smooth muscle cells treated with oxidized LDL and $ApoE^{-/-}$ mice (model for atherosclerosis), the application of hydralazine and its derivatives was reported to block the carbonyl stress through reversing the decrease of free amino group contents and the increase of carbonylated protein levels [97]. All these studies suggest the beneficial effects of hydralazine and its derivatives as ACR scavengers. In some other reports, however, their beneficial effects were found to have a poor correlation with their ACR scavenging capacity. For instance, naphthylhydrazine, a weak scavenger showed strong cytoprotective potency, while dihydralazin, a powerful scavenger only exhibited moderate cytoprotection, evidenced from the results of half-maximal protection against cell death in hepatocytes induced by ally alcohol. Some other factors should also be taken into account such as cell membrane permeability of difference scavengers, and their effects towards other intracellular targets involved in cell survival and death [11].

Carnosine (β-alanyl-L-histidine), together with some structurally related dipeptides, belongs to a group of histidine-containing molecules that widely distribute in vertebrate organisms, especially in muscles and nervous tissues [98]. It is believed that carnosine and its related dipeptides function as antioxidants, free radical scavengers [99, 100], metal chelators (copper and zinc) [101] and neurotransmitters [102]. Carnosine was also demonstrated to possess anti-aging properties [103]. Recently, caronsine was reported to attenuate methylglyoxal-induced protein cross-links [104, 105] and acetaldehyde/formaldehyde-induced DNA–protein cross-links [106]. In an RNase A system, ACR was found to induce oligomerization of RNase A, while at the concentration of 1 and 3 mM, carnosine inhibited the formation of RNase A oligomers. However, the effect of carnosine on the RNase A oligomerization was less striking compared with hydralazine and GSH [91]. In another study with protein aggregation induced by ACR in neurofilaments (a major element of the neuronal cytoskeleton), carnosine, homocarnosine and anserine were found to significantly lessen the aggregation by diminishing the formation of dityrosine, protein carbonyl compounds and amyloid-like characteristics [107]. All these studies suggested the beneficial effects of carnosine derivatives as ACR scavengers.

Aminoguanidine has been known as an inhibitor against the formation of advanced glycation end products (AGEs) for a long time. It is a well-studied scavenger of dicarbonyls such as methylglyoxal (MGO), GO and 3-deoxyglucosone (3-DG) [108]. However, little information is available about its trapping efficacy against ACR. Only recently, aminoguanidine was reported to exhibit weaker effects than cysteine to block the loss of activity caused by ACR in human PON-1 and human AT [53, 54]. Another issue making aminoguanidine less attractive as an ACR scavenging is its serious adverse effect in clinical trials. It is suspected to trap pyridoxal, leading to vitamin B6 deficiency and neurotoxicity in long-term administration [109].

Pyridoxamine, together with pyridoxine and pyridoxal, is a natural form of vitamin B6. It is reported as a potential drug candidate against protein glycation via multiple mechanisms such as reacting with Amadori compounds that act as precursors of AGEs, and chelating metal ions that catalyze Amadori reactions [110]. Pyrodoxamine has also been well known as a scavenger of several aldehydes generated from lipid peroxidation including MDA, GO and glycolaldehyde (GLA) [111]. It is suggested that PM reacts primarily with some early intermediates or precursors of GO, MGO, MDA and HNE in the AGEs and/or advanced lipid peroxidation end products (ALEs) formation in vivo [112]. Several pyridoxamine adducts formed with some intermediates of lipid peroxidation have already been identified in the urine of pyridoxamine-treated animals [113]. In addition, clinical investigation of pyridoxamine as a therapeutic agent in the treatment of diabetic nephropathy is now under way [114], casting light on the research of pyridoxamine as an inhibitor of ACR-induced carbonyl stress.

4 Naturally occurring phenolic compounds as ACR scavengers

4.1 Reactivity

Some polyphenols recently have been investigated as direct trapping agents of dicarbonyls (GO and MGO) [115–117]. Similar polyphenols have also been documented as effective trapping agents of ACR [118–120]. In a screening of 21 natural polyphenols, it was found that nine of them (1 mM) directly quenched ACR (0.5 mM) by 27.4–99.6% under simulated physiological conditions (0.01 M PBS, pH 7.4, 37°C) with 90 min incubation. These effective polyphenols are epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate (EGCG), theaflavin, theaflavin-3,3′-digallate, cyanomaclurin, phloretin and phloridzin, of which phloretin is the most powerful scavenger (Fig. 5). Examination of the structure–activity relationship showed that a phloroglucinol moiety (usually called an A ring in these structures) was shared among all the effective scavengers, suggesting the importance of this skeleton for trapping of ACR [119]. Furthermore, substitution of any of the hydroxyl groups in A ring weakened the activities for electrophilic aromatic substitution, therefore lowering the trapping effects [120].

4.2 Mechanism

The Michael addition reaction may be a major mechanism to explain why polyphenols have emerged as a novel class of ACR scavengers. The reaction between ACR and some polyphenols has been systematically studied. The adducts of 1:1 and 1:2 EGCG/ACR were first detected when ACR was incubated with EGCG [118, 119], which clearly showed that EGCG can directly trap ACR. Further confirmative evidence is from the research to evaluate ACR-trapping capacities of the A, B and C rings of EGCG by SPME-GC-MS analysis. Phloroglucinol (A ring analogue of EGCG) was found to completely trap ACR in 0.01 M PBS (pH 7.4, 37°C), indicating that phloroglucinol ring is indispensible for ACR scavenging [119]. Finally, conclusive information was obtained from the complete structural elucidation of the di-ACR-conjugated phloretin adduct by NMR analysis. Based on the structure of the adduct, the chemical rationale for the probable nature of interactions between ACR and phloretin is proposed as followed (Fig. 6): Phloretin likely undergoes Michael addition to the C=C double bond of ACR, which mainly involves C-3 and/or C-5 of the A ring of phloretin. Subsequently, nucleophilic attack at the terminal aldehyde by a nearby hydroxyl group at C-2 and/or C-4 leads to the formation of cyclic hemiacetal(s) as more stable final products [120]. With the understanding of chemical structures of the formed adducts and the reaction mechanisms, more and more potential natural polyphenols possessing a similar phloroglucinol ring can be suggested as ACR scavengers in the future.

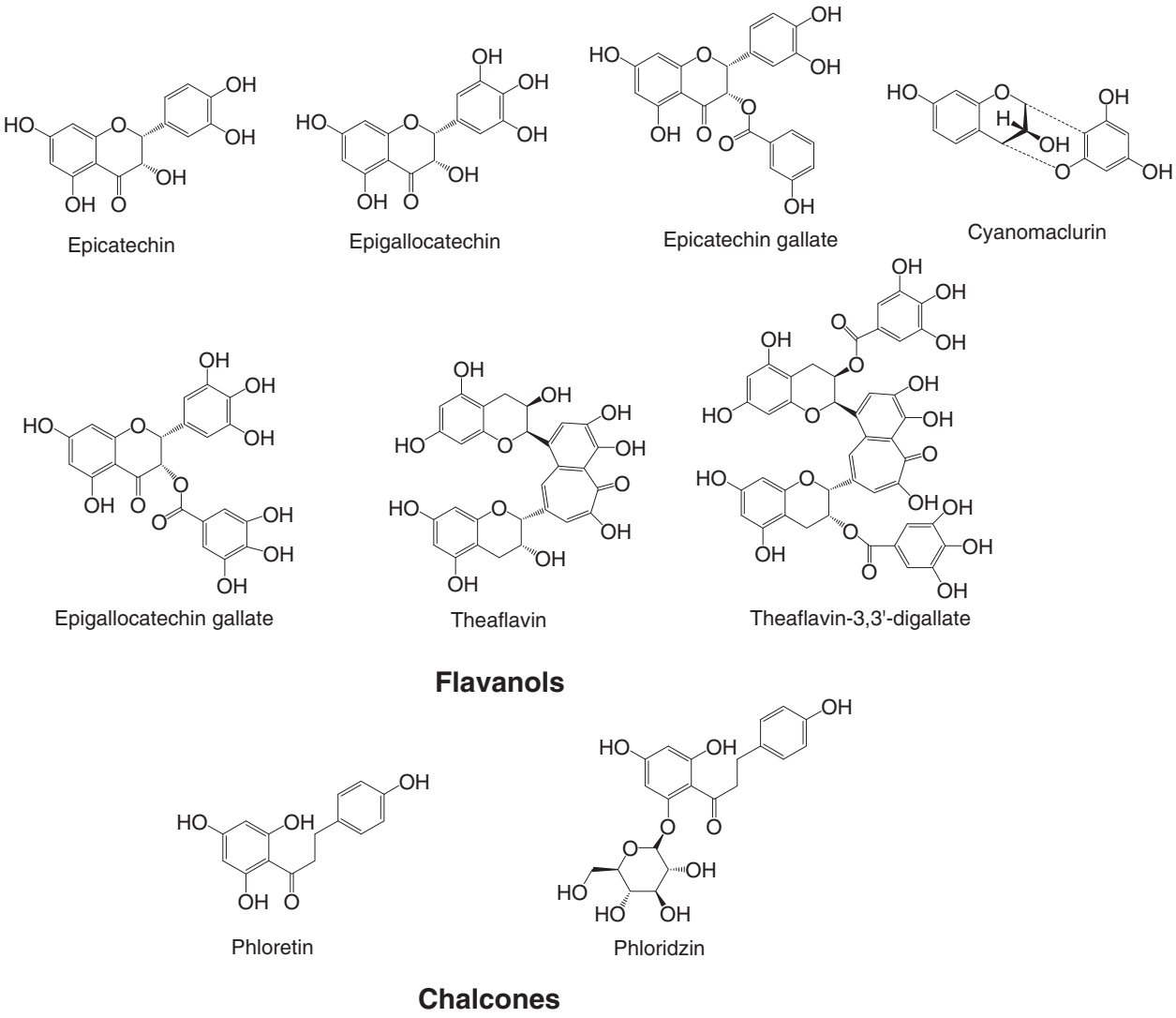

Figure 5. The chemical structures of several naturally occurring polyphenols as acrolein scavengers.

4.3 Impact on health

The ubiquitous existence in many plants and the role as an integral part of human diet make natural polyphenolic compounds good candidates for promoting human health. Polyphenolic compounds were traditionally used as antioxidants, terminators of free radicals (such as oxygen ions and peroxides) and chelators of metal ions [121]. The recent emergence of polyphenols' new role as ACR scavengers might provide more information in understanding the complicated interaction between polyphenols and the cascade of intermediates formed during lipid peroxidation as well as the end products such as α,β-unsaturated aldehydes, which also suggests multiple roles of polyphenols in the management of human health. It was reported recently that some polyphenols can lower the cellular toxicity of ACR in several cell cultures. For example, pycnogenol (PYC), a patented combination of polyphenols extracted from French maritime pine (*Pinus maritima*) bark can markedly defense against ACR-induced toxicity in human neuroblastoma SH-SY5Y cells. The intracellular GSH levels, ROS production and NADPH oxidase activation were all modulated in a dose-response manner by PYC, resulting in higher cell viability, reduced protein oxidation/nitration (protein carbonyl, 3-nitrotyrosine) and inhibition of the formation of a major lipid peroxidation product, HNE [122]. *Bacopa monniera* (BM) extract, one of the most important medicinal plants used in Indian traditional medicine for enhancing memory and cognition was also reported to show beneficial effects against ACR-induced toxicity in cell cultures. In addition to the inhibition of ROS generation in human neuroblastoma cells, BM treatment also influenced the mitochondrial membrane potential and affected the expression of several redox regulation proteins such as NF-κB, Sirt1,

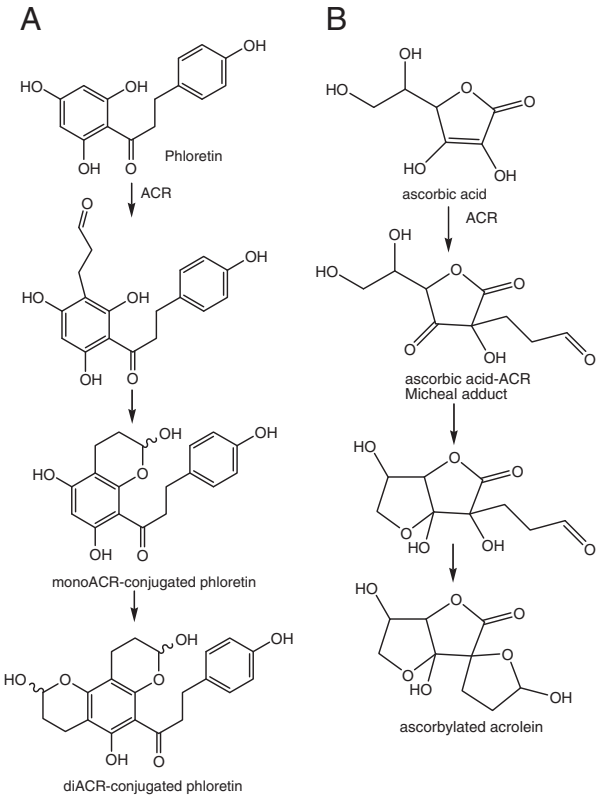

Figure 6. The proposed reaction acrolein scavenging mechanism by phloertin (A) and ascorbic acid (B).

ERK1/2 and p66Shc, leading to a better defense against ACR [123]. Furthermore, a polyphenol-riched *Scutellaria baicalensis* Georgi (Labiatae) extract was demonstrated to lower the cytotoxicity of ACR in human umbilical vein endothelial cells via increasing GSH content and upregulating the mRNA expression of GSH synthesis enzymes [124]. Moreover, a recent study demonstrated that decaffeinated green tea extract can attenuate cooking-oil-fumes-induced acute lung injury through the suppression of cooking oil fume-induced upregulation of ROS level and the downregualtion of anti-apoptosis and chaperone protein in rats [125]. As ACR is a major reactive carbonyl compound in cooking oil fumes, this research may suggest the beneficial effects of green tea polyphenols against ACR-induced toxicity.

In addition to polyphenol-riched extracts, pure polyphenolic compounds have also been evaluated for their effects on ACR-induced toxicity. For instance, the most abundant tea catechin, EGCG was reported to block the formation of ACR-derived Acr-dG when dG was incubated with ACR or DHA under oxidative condition [76]. Robinetinidol-(4β→8)-epigallocatechin 3-*O*-gallate (REO) isolated from *Acacia mearnsii* De Wild was recently investigated in ACR-challenged SH-SY5Y cells. Results showed that it functioned similarly as PYC and exhibited additional anti-apoptotic actions as well, through the reversal of enhanced caspase-3 activation and the suppression of an apoptotic mediator phospho-JNK triggered by ACR [126]. EGCG was also demonstrated to be effective in the same study. However, inconsistent results were reported in a study with mouse mammary carcinoma FM3A cells, where tea polyphenols containing 35.3% of EGCG as well as 63.1% of other catechins failed to protect the cells against ACR-induced toxicity [55]. Resveratrol, a red wine polyphenol, was reported to significantly improve the phagocytic function of human RPE cells in acute ACR and combined ACR/H_2O_2-induced toxicity [127]. Hydroxytyrosol, an abundant phenolic compound in olives or virgin olive oil, was found to effectively ameliorate the oxidative damage and mitochondrial dysfunctions induced by ACR in ARPE-19 human RPE cells [128]. In the subsequent study, hydroxytyrosol's modulatory effects on Nrf2 and peroxisome proliferator-activated receptor coactivator 1 α (PPARGC1α) were demonstrated to be responsible for its health benefit.

Part of the beneficial effects mentioned above for natural polyphenols might be attributed to their ACR trapping capacities. However, it is still difficult to draw a conclusion that there is a positive correlation between them, as there is limited information about the direct interaction of phenolics with ACR in complicated biological systems and polyphenol–ACR adducts have not been detected in vivo.

Furthermore, recent studies suggest that ACR is not only a product generated in lipid peroxidation but also a possible initiator in oxidative stress. The attenuation of ACR-induced toxicity by polyphenols may also be related to their anti-oxidative properties, as well as induction of elevated GSH levels and activation of antioxidant enzymes. Therefore, the real role of polyphenols is hard to define under the circumstance of oxidative stress. However, this should not prevent the public from continuous consumption of dietary polyphenol-riched food products as dietary polyphenols have been associated with the prevention of certain chronic diseases, such as cancers, cardiovascular and neurodegenerative diseases [129].

5 Ascorbic acid as an ACR scavenger

Ascorbic acid (vitamin C) is mentioned specifically in this review for its role as a recognized Michael donor to ACR. It can form conjugates with ACR in vitro through one reaction entitled as ascorbylation of ACR [130]. According to Stevens and Maier [12], ascorbic acid can react with ACR through Michael addition with further cyclization to form a 5,5,5-tricyclic spiro-compound (AscACR) in aqueous solvents (Fig. 6). In biological system, AscACR was found to be further metabolized to 5,6,7,8-tetrahydroxy-4-oxooctanal (THO) and THO was detected in cultured human monocytic THP-1 cells with the formation facilitated by the lactonase enzymes [131]. Given the high intracellular concentration of ascorbic acid in humans (in the low millimolar range) [132], ascorbic acid might be a

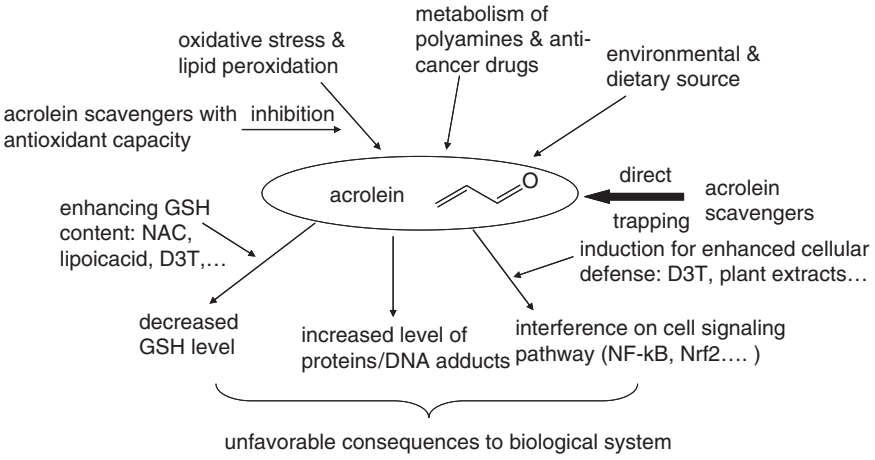

Figure 7. Schematic presentation of proposed mechanisms of protective effects of acrolein scavengers on acrolein-induced toxicity.

promising agent for detoxification of ACR in a cellular environment. The direct conjugation of ascorbic acid with ACR might be implicated in ascorbic acid's reduction of the modification of apolipoprotein E in human very LDL [133]; preservation of functional and anatomical parameters in isolated spinal cord white matter [134] and inhibition of apoptosis in human bronchial epithelial cells induced by ACR [135].

6 Concluding remarks

Several classes of natural products/synthetic compounds have shown great potential as ACR-trapping agents. They have different reactivity towards ACR through different trapping mechanisms. As shown in Fig. 7, apart from direct trapping effects, these ACR scavengers exhibited multiple functions, ranging from the upstream inhibition of lipid peroxidation to the downstream attenuation of cellular damage caused by ACR. Alterative ways to reduce the toxicity of ACR are also possible, such as the activation of GST enzymes that facilitates the conjugation of ACR to GSH, the enhancement of carbonyl metabolism through manipulation of enzymes related to reduction of reactive aldehydes. In addition, the latest findings have pointed out that ACR might act as an initiator of oxidative stress other than a product [136]. Therefore, the strategies for the inhibition of damaging consequences caused by oxidative stress could also shed light on the counteraction of ACR-associated toxicity.

The studies of ACR scavengers represent an emerging field of interest in the development of potential agents for disease prevention and therapy. However, a lot of issues have to be addressed before the application for this purpose. In future studies, the scavenging mechanisms in real biological systems have to be elucidated and confirmed. The fate of adducts generated from ACR scavenging also needs to be considered: do they degrade or accumulate in tissues and will they pose other problems in metabolism? Meanwhile, the investigation of the metabolic profiles of these scavengers in human body is required to ensure their safety.

The authors have declared no conflict of interest.

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