molecules

MDPI

Review

Targeting Reactive Carbonyl Species with Natural Sequestering Agents

Sung Won Hwang [1], Yoon-Mi Lee [2,3], Giancarlo Aldini [4] and Kyung-Jin Yeum [2,3,*]

1 Department of Nano Science & Mechatronics Engineering, College of Science and Technology, Konkuk University, Chungju-si 27478, Korea; swhwang@kku.ac.kr
2 Division of Food Bioscience, College of Biomedical and Health Sciences, Konkuk University, Chungju-si 27478, Korea; yoonmilee@kku.ac.kr
3 Interdisciplinary Research Center for Health, Konkuk University, Chungju-si 27478, Korea
4 Department of Pharmaceutical Sciences Pietro Pratesi, Università degli Studi di Milano, via Mangiagalli 25, Milan 20133, Italy; giancarlo.aldini@unimi.it
* Correspondence: kyeum@kku.ac.kr.com; Tel.: +82-43-840-3586; Fax: +82-43-840-3585

Academic Editors: Maurizio Battino and Etsuo Niki
Received: 3 January 2016; Accepted: 23 February 2016; Published: 27 February 2016

Abstract: Reactive carbonyl species generated by the oxidation of polyunsaturated fatty acids and sugars are highly reactive due to their electrophilic nature, and are able to easily react with the nucleophilic sites of proteins as well as DNA causing cellular dysfunction. Levels of reactive carbonyl species and their reaction products have been reported to be elevated in various chronic diseases, including metabolic disorders and neurodegenerative diseases. In an effort to identify sequestering agents for reactive carbonyl species, various analytical techniques such as spectrophotometry, high performance liquid chromatography, western blot, and mass spectrometry have been utilized. In particular, recent advances using a novel high resolution mass spectrometry approach allows screening of complex mixtures such as natural products for their sequestering ability of reactive carbonyl species. To overcome the limited bioavailability and bioefficacy of natural products, new techniques using nanoparticles and nanocarriers may offer a new attractive strategy for increased *in vivo* utilization and targeted delivery of bioactives.

Keywords: reactive carbonyl species; natural products; bioefficacy; nanotechniques

1. Introduction

Reactive oxygen species (ROS) are continuously generated through normal cell metabolism in the body [1], and are necessary for biological homeostasis [2]. However, an imbalance between oxidant production and antioxidant defense can lead to an accumulation of excess ROS which damage vulnerable targets such as unsaturated fatty acids in membranes, thiol groups in proteins and nucleic acids in DNA [3]. Thus, oxidative stress can be associated with the development and progression of various chronic diseases. In particular, elevated cytotoxic reactive carbonyl species, which are produced by the oxidation of polyunsaturated fatty acids and sugars [4], plays a crucial role in the progression of metabolic disorders such as diabetes [5] and cardiovascular diseases [6] and neurodegenerative diseases [7]. Carbonyl species are highly reactive due to their electrophilic nature, and easily react with the nucleophilic amino acids such as Lys, His and Cys, leading to the formation of protein adducts [8,9]. The formation of these protein adducts has been reported to cause irreversible cellular dysfunction [10,11].

The use of natural products that can effectively sequester reactive carbonyl species [12,13] may offer a novel strategy blocking the pathological conditions and progression of various chronic diseases.

In addition, new techniques such as nanoparticles and nanocarriers that can increase the bioavailability and bioefficacy of natural products *in vivo* may open up a field for preventing oxidative stress associated chronic diseases using natural products.

However, substantial knowledge gaps still exist including: (1) what effective sequestering agents are and their mechanisms *in vivo*; (2) reactive carbonyl species etiology leading to cellular dysfunction *in vivo*; and (3) whether genetic variation affects the biological efficacy of different sequestering agents for reactive carbonyl species.

2. Oxidative Stress and Reactive Carbonyl Species

Reactive carbonyl species can be classified into three groups: (1) α,β-unsaturated aldehydes such as 4-hydroxy-*trans*-2-nonenal (HNE) and acrolein; (2) keto-aldehydes such as methylglyoxal and (3) dialdehydes such as glyoxal and malondialdehyde as shown in Figure 1 [14].

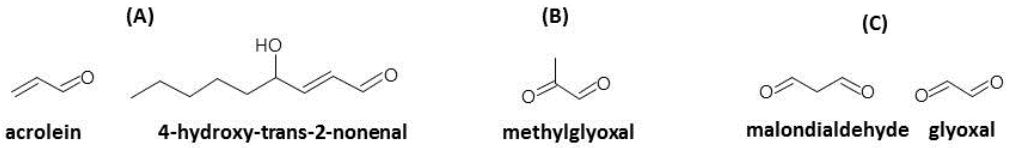

Figure 1. Structures of reactive carbonyl species: α,β-unsaturated aldehydes (**A**); keto-aldehyde (**B**) and di-aldehydes (**C**).

Proteins represent the most studied target of reactive carbonyl species and the corresponding reaction products are named advanced glycation end products (AGEs) when the attacking RCS is derived from sugar, and called advanced lipoxidation end products (ALEs) when it derives from lipids. AGEs and ALEs share similar structural and biological properties. For example, both consist of non-enzymatic, covalently modified proteins and oxidative stress is often (but not always) involved in the mechanism of their formation. Moreover some AGEs and ALEs have the same structure, since they arise from common precursors, as in the case of carboxymethyllysine (CML) which is generated by glyoxal that is formed by both lipid and sugar oxidative degradation pathways [14].

HNE represents one of the most abundant and toxic reactive carbonyl species, which is generated via β-cleavage of hydroperoxide derived from ω-6 polyunsaturated fatty acids such as linoleic acid and arachidonic acid [4]. HNE can give covalent adducts with the protein nucleophilic side chains, namely, the cysteine thiol group, the lysine ε-amino group, and the histidine imidazole ring [15]. AGEs are generated by the covalent reaction of reactive carbonyl species derived from sugar oxidation such as glyoxal, methylglyoxal and 3-deoxyglucosone with the nucleophilic protein sites, as well as by the condensation of the carbonyl group of reducing sugars with the primary amino group of the lysine side chain or of the protein N-terminus [16]. Covalent modifications of AGEs and ALEs can induce a functional disorganization of proteins since covalent modification causes the protein to undergo a conformational change, undergo catalytic site distortion or impairment of the function of the protein itself. AGEs and ALEs can further modify proteins by inducing signal transduction leading to cellular damage [16]. The interaction of AGEs and possibly ALEs with receptors for advanced glycation end products (RAGE) leads to NFκB activation which is known to cause the production of inflammatory cytokines including IL-1, IL-6 and TNF-α. RAGE has even been proposed as a master switch to turn on the proinflammatory response into a cellular dysfunction [17]. RAGE activation also induces the production of excessive mitochondrial ROS thereby leading to mitochondrial superoxide accumulation [18]. In that sense, it is reasonable to consider that blocking ROS production is an appropriate strategy in order to reduce mitochondrial superoxide accumulation in diabetic patients [18]. It is interesting to note that an elevation of keto-aldehydes such as methylglyoxal in type II diabetic patients, and its reduction by the diabetic drug, metformin, has been observed [19]. In addition, methylglyoxal has been reported to play a critical role in diabetic complication, nephropathy [20].

The association of AGEs/ALEs with chronic diseases and the mechanism of interaction between AGEs and RAGE have been identified partially. However, effective AGEs/ALEs sequestering agent which can block the AGEs-RAGE interaction, and its ability to inhibit inflammatory responses and related pathologic progression of chronic diseases *in vivo*, is not yet known.

3. Implication of Reactive Carbonyl Species on Metabolic Disorders

Evidence is mounting that oxidative stress and protein carbonylation damage induced by reactive carbonyl species are involved in metabolic disorders such as dyslipidemia, insulin resistance, vascular and renal diseases [10,21–23]. Table 1 presents targets of different reactive carbonyl species to prevent metabolic disorders in cells, animal models and humans. Various cell lines such as muscle cells [24], pancreatic β-cells [25] and human mesangial cells [26] have been studied to determine various drug actions to block reactive carbonyl species, AGEs, RAGE and protein carbonyls, thereby preventing metabolic disorders. In addition, various animal models utilized to determine the effect of blocking reactive carbonyl species, AGEs and RAGEs on metabolic disorders and their complications. Zucker rats [27] and ApoE null mice [28] have been employed to evaluate the reactive carbonyl species sequestering actions of carnosine and its derivatives, respectively. In addition, streptozotocin induced diabetic rats [29], CCl_4-injected [30] or high fructose-fed [31] Wistar rats, and methylglyoxal injected Dahl salt-sensitive rats [32] were used to target reactive carbonyl species, AGEs, RAGE and/or protein carbonyls in diabetic complications. Furthermore, in humans, type II diabetic patients were studied for their elevated methyl glyoxal levels [19] as well as RAGE expression [33]. Nonetheless, reactive carbonyl species and their adducts are closely associated with the progression of metabolic disorders and such complications and can be alleviated by reactive carbonyl species sequestering agents.

Table 1. Studies targeting reactive carbonyl species to prevent metabolic disorders.

Metabolic Disorders	Targeting RCS	Tested Agent	Model	Ref.
Cell Studies				
Insulin resistance	HNE, Protein carbonyls	D3T, NAC, AGD, SAM	Gastrocnemius muscle, muscle cells (L-6)	[24]
Insulin resistance	AGEs, Protein carbonyls	AGD, Pyridoxamine	Pancreatic β-cells (HIT-T15)	[25]
Diabetic nephropathy	RAGE	Glucagon-like peptide 1	Human mesangial cells	[26]
Animal Studies				
Dyslipidemia	HNE, AGEs	Carnosine	Zucker Fa/Fa rats	[27]
Atherosclerosis Renal disease	HNE, ALEs	D-carnosine octylester	ApoE null mice (HFD)	[28]
Diabetic atherosclerosis	RCS, AGEs, ALEs, RAGE	LR-90	Streptozotocin induced diabetic rats	[29]
Liver damage	AGEs, RAGE, protein carbonyls	Glycyrrhizi	High fructose-fed Wistar rats	[31]
Liver/renal toxicity	RAGE, Protein carbonyls	Peach	CCl_4 injected Wistar rats	[30]
Diabetic nephropathy	RAGE	Candesartan	MG injected Dahl salt-sensitive rats	[32]
Human Studies				
Diabetes and complication	RAGE	Simvastatin	Type 2 diabetic patients	[33]
Diabetes	MG	Metformin	Type 2 diabetic patients	[19]

RCS, reactive carbonyl species; HNE, 4-hydroxy-trans-2-nonenal; D3T, 3*H*-1,2-dithiole-3-thione; NAC, *N*-acetyl-cysteine; AGD, aminoguanidine; SAM, *S*-adenosylmethionine; AGEs: advanced glycation end products; RAGE, receptor for advanced glycation end products; ALEs, advanced lipoxidation end products; HFD, high fat diet; MG, methyl glyoxal.

4. Implication of Reactive Carbonyl Species on Neurodegenerative Diseases

The carbonylation of histidine and lysine residues of apolipoprotein B (apoB-100) in low-density lipoproteins (LDL) has been reported to be implicated in the formation of foam cells [34]. Interestingly, modified LDL by HNE has also been found to cause a significant elevation of β-amyloid

fibrillogenesis [7], suggesting an involvement of reactive carbonyl species in neurodegenerative diseases such as Alzheimer's disease. Table 2 lists several *in vitro* and *in vivo* studies targeting reactive carbonyl species for preventing neurodegenerative diseases.

Table 2. Studies targeting reactive carbonyl species to prevent neurodegenerative diseases.

Neurodegenerative Diseases	Targeting RCS	Tested Agent	Model	Ref.
Cell Studies				
Neuronal damage	MDA, AGE-RAGE, Protein carbonyls	EGCG	AGE treated SH-SY5Y cells	[35]
Neuronal damage	MDA, Protein carbonyls	Notoginsenoside	H_2O_2 treated PC12 cells	[36]
Animal Studies				
Brain inflammation	AGEs, RAGE, Protein carbonyls	Ursolic acid	D-galactose injected Kunming mice	[37]
Neuronal damage	MDA, Protein carbonyls	Melatonin	^{56}F-irradiated C57BL mice	[38]
Alzheimer's disease	AGEs, Protein carbonyls	Troxerutin	High cholesterol fed C57BL/6 mice	[39]
Alzheimer's disease	HNE	Antisense oligonucleotide	SAMP8 mice	[40]
Alzheimer's disease	HNE, Protein carbonyls	Curcumin	Streptozotocin-injected Wistar rats	[41]
Alzheimer's disease	HNE, Protein carbonyls	Ferulic acid ethyl ester	AAPH or Fe^{2+}/H_2O_2 injected Mongolian gerbils	[42]
Human Studies				
Cognitive dysfunction	HNE, Protein carbonyls	2-Mercaptoethane sulfonate	doxorubicin-received patients	[43]

RCS, reactive carbonyl species; AGEs: advanced glycation end products; RAGE, receptor for advanced glycation end products; EGCG, epigallocatechin gallate; AAPH, 2,2′-Azobis(2-amidinopropane) dihydrochloride; HNE, 4-hydroxy-trans-2-nonenal.

Reactive carbonyls species and protein carbonyls have been reported to induce neuronal damage, and bioactives such as epigallocatechin gallate [35] as well as notoginsenoside [36] alleviated such damage in neuronal cell lines. In addition, various studies utilizing animal models also presented consistent results. Animal studies utilizing D-galactose-injected C57BL/6 mice [37], ^{56}F-irradiated C57BL mice [38], high cholesterol-fed C58BL/6 mice [39], SAMP8 mice [40], streptozocin-injected Wistar rats [41], 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) or Fe^{2+}/H_2O_2-injected Monglian gerbils [42] indicated involvement of reactive carbonyl species and protein carbonyls on neuronal damage and Alzheimer's disease. These studies indicated that such diseases were ameliorated by blocking oxidative damaged caused by reactive carbonyl species. In cancer patients, mercaptoethane sulfonate has been reported to be used for reducing oxidative stress induced by doxorubin treatment [43].

Unfortunately, an effective preventive strategy for chronic diseases such as metabolic disorders and neuronal diseases is currently lacking. However, identification of natural products that are able to directly or indirectly detoxify the reactive carbonyl species may offer new therapeutic agents to combat such diseases. The hypothetical sequestering mechanism of natural products for cytotoxic reactive carbonyl species is presented in Figure 2.

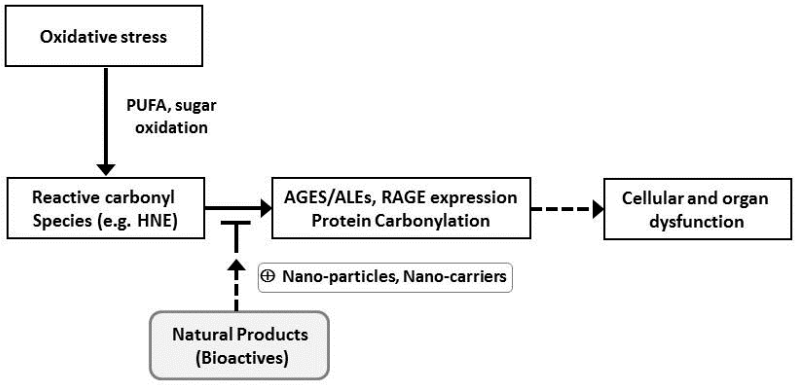

Figure 2. Hypothetical cytotoxic reactive carbonyl species sequestering action of natural products. PUFA, polyunsaturated fatty acid; HNE, 4-hydroxy-*trans*-2-nonenal; AGEs/ALEs, advanced glycation end product/advanced lipoxidation end products; RAGE, receptor for advanced glycation end products.

5. Analytical Techniques for Identifying Reactive Carbonyl Species Sequestering Agents

In order to determine the sequestering actions of natural products on reactive carbonyl species, a reliable and accurate method that can identify these actions of bioactives in complex mixture is required. Several approaches have been reported for identifying such compounds as shown in Table 3.

Table 3. Analytical techniques identifying reactive carbonyl species sequestering agents.

Analytical Techniques	Advantages	Disadvantages	Ref
Spectrophotometry	Simple Fast	No specificity No application for complex mixture	[44]
HPLC	Limited specificity	No application for complex mixture Produce by-product	[45]
NMR spectroscopy	Molecule identification	No qunatitation Expensive Require large quantity of sample	[46]
Western blot	Semiqunatitative	Time consuming	[47]
LC-MS	Quantitative Complex mixture analysis	Molecule identification require further analysis	[48]

HPLC, high performance liquid chromatography; NMR spectroscopy, nuclear magnetic resonance spectroscopy; LC-MS, high resolution mass spectrometry.

A spectrophotometric assay has been widely used to analyze chromophore containing reactive carbonyl species such as α,β-unsaturated aldehydes directly or through a derivatization process for unconjugated reactive carbonyl species such as malondialdehyde, glyoxal, and methylglyoxal [44]. The reactive carbonyl species quenching activity can be determined by the disappearance of aldehyde in the presence of a compound of interest. An integration of HPLC with UV analysis was also utilized to increase specificity [45]. However, these types of approaches cannot be applied to mixed compounds containing natural products. In addition, by-products can be produced in the process of sample preparation resulting in the loss of accuracy.

Determination of the formation of AGEs/ALEs by incubating reactive carbonyl species with a target protein in the presence of a potential quencher has also been used to identify sequestering agents against reactive carbonyl species. The formation of AGEs/ALEs can be determined by increased molecular weight using NMR spectroscopy [46] or Western blot [47]. However, these types of assay can be time consuming, expensive and cannot be quantitative.

A new approach using high resolution mass spectrometry was reported to test the ability of natural compounds inhibiting protein carbonylation induced by reactive carbonyl species [48]. It consists of incubating ubiquitin with 4-hydroxy-*trans*-2-nonenal (HNE), in the presence and absence of natural products. After incubation, the reaction can be stopped and analyzed for reaction metabolites using high-resolution mass spectrometry. This approach has been validated by measuring the effect of well-known reactive carbonyl species sequestering agents, such as aminoguanidine, pyridoxamine, hydralazine and carnosine. A highly reproducible mass spectrometric method was also found suitable for testing reactive carbonyl species sequestering ability of complex mixtures such as plant extracts, thus furnishing a methodological approach for identifying novel natural compounds that are effective as reactive carbonyl species sequestering agents. It should be noted that an approach permiting evaluation of overall quenching activity of complex mixtures open limits identification of responsible component(s) for the quenching activity. Characterization of sequestering agent in natural products require further analysis coupled with informatics approach.

6. Reactive Carbonyl Species Sequestering Actions of Natural Products

Convincing evidence is accumulating that a higher consumption of fruits and vegetable reduces all-cause mortality and cardiovascular mortality [49], whereas no beneficial [50,51] and even harmful effects [52,53] of multivitamins or antioxidant supplements against chronic diseases has been observed. Considering several natural products have been reported for their reactive carbonyl species sequestering action, natural products that can effectively sequester reactive carbonyl species can be a potential preventive strategy against such chronic diseases.

6.1. Histidine-containing Dipeptides

In vitro studies have shown that histidine dipeptides such as carnosine (β-alanyl-L-histidine) and anserine (β-alanyl-L-methylhistidine) effectively detoxifies HNE by forming unreactive adducts [54]. Notably, histidine, which is one of the most reactive nucleophilic residues in protein, is a primary reaction site of HNE adduction [55]. Histidine-dipeptides such as carnosine supplementation has been reported to significantly reduce the development of dyslipidemia, hypertension and renal injury by reducing the extent of protein carbonylation and glycation in Zucker obese rats [27]. In addition, histidine-dipeptides have proven to be beneficial in various animal models characterized for systemic oxidative and/or glycative stress [27,56–60]. There is also compelling evidence that histidine-dipeptides mediate their health-promoting effects by decreasing the levels of AGEs/ALEs thereby blocking damage of AGEs/ALEs-RAGE in these animal models.

Gene-nutrient interactions may result in different bioefficacy of supplements according to the genetic background of individuals. Such interactions have been reported in vitamin C-glutathione S-transferase [61] and vitamin E-haptoglobin [62]. The association of low serum carnosin concentration with diabetic nephropathy has also been reported. It was found that carnosinase encoding gene, CNDP1, linked with the late onset of complications for people with diabetes [56,63]. More specifically individuals who have the 5-6, 5-7, 6-6, and 6-7 alleles of the CNDP1 gene had elevated serum carnosinase activity. Diabetic patients with the 5-5 allele, which accounted for about 1/3 population in this study, were found to be less susceptible to renal complication [56,63]. It is reasonable that the higher expression of carnosinase increases carnosine degradation leading to a lesser degree of renal protection by carnosine. Although such a hypothesis should be verified further in human studies, nutrient-gene interaction is an area needs to be explored for the understanding of bioefficacy of natural products.

6.2. Plant Products

More recently, black rice with giant embryos rich in GABA, anthocyanin, γ-oryzanols, α-tocopherol and α-tocotrienols has been reported to suppress hyperlipidemic and hyperinsulinemic responses in *ob/ob* mice [64]. Although one should be cautious when extrapolating results from

animal studies to humans, identification of such activity in natural products can provide more targeted preventive strategy against chronic diseases. In addition, the effects of green coffee bean extract and procyanidins from *Vitis vinifera* on protein carbonylation have been demonstrated using the newly developed mass spectrometry approach [48]. These two extracts are reported to have an effective inhibition of HNE induced ubiquitin carbonylation in a dose-dependent manner *in vitro*.

Screening of natural products for reactive carbonyl species quencher is the first step to identify potential candidates for a targeted strategy preventing oxidative stress associated chronic diseases. However, several further steps need to be made including understanding of gene-nutrient interactions, and increasing the limited bioavailability and bioefficacy of natural products.

7. Nanotechnologies for Bioavailability and Bioefficacy of Natural Products

The major hindrance of oral intake of natural products, including phytochemicals, is their limited bioavailability due to their poor solubility, instability, and negligible intestinal absorption. Considering mega-doses is not a solution to address the limited bioavailability of such natural products, so development of effective delivery systems improving bioavailability and bioefficacy is a key issue for nutraceutical research. In fact, applications of nanotechnology to improve bioavailability and bioactivity of diet-derived phytochemicals have been reported recently [65–67]. Biocompatible and biodegradable nanoparticles such as nanoemulsions, nanoliposomes, and nano-carriers are reported to resolve the limited bioavailability of phytochemicals, as summarized in Figure 3 [65].

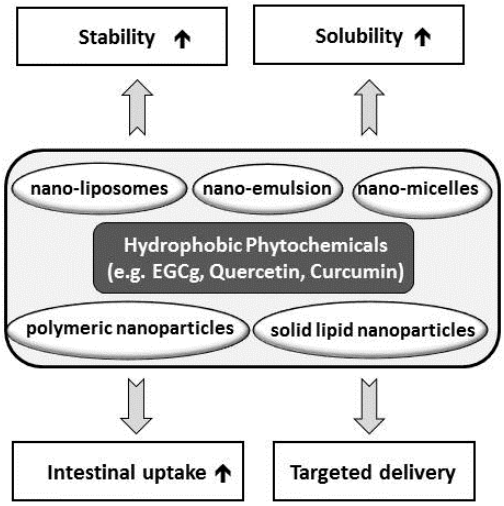

Figure 3. Application of nano-technologies improving bioavailability of natural products. EGCg, epigallocatechin gallate.

It has been reported that nanoliposomes enhanced the stability of epigallocatechin gallate (EGCG) [68], and increased its antioxidant activity [69]. Nanomicelles were applied to overcome the low bioavailability of quercetin, which is a plant-derived hydrophobic flavonol [70,71]. In addition encapsulation of quercetin is reported to maintain free quercetin levels in blood and target tissues by delaying its metabolism [72]. Oral bioavailability [73] and its bioefficacy [74] of hydrophobic curcumin has also been reported to be dramatically improved by application of nanotechnology.

In addition, advances in technology for nanomaterials [75,76] may also provide great potentials for improving bioavailability and bioefficacy of natural products. The recent discovery of graphene has spurred on various research approaches for targeted delivery of active compounds. Graphene is a single atom thick layer of sp^2-hybridized carbon atoms arranged in a honeycomb two dimensional (2D) crystal lattice [77]. Owing to its unique atomic structure, graphene has flexible physical and chemical properties, large surface area and biocompatibility, fast mobility and outstanding electrical conductivity [78]. These properties make graphene an ideal material for a variety of applications

including quantum mechanics and engineering of biomaterials such as new generation biosensors [79], probes for biological imaging [80] and nanocarriers for drug delivery [81]. Among various nano-materials explored for the last two decades for drug delivery, graphene, graphene oxide (GO) and grapheme quantum dots (Figure 4) have emerged as new competitive nanocarriers for drug delivery and possibly natural products delivery.

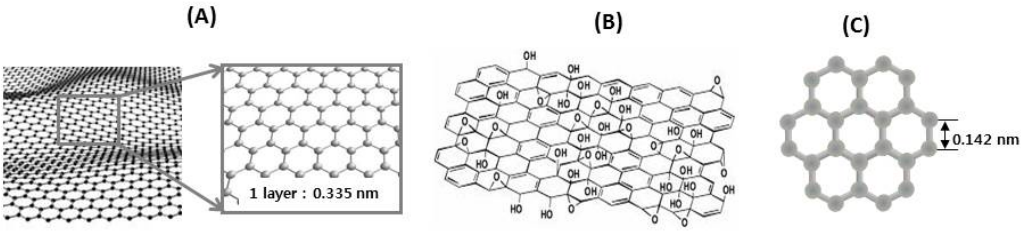

Figure 4. Structures of graphene (**A**); graphene oxide (**B**) and grapheme quantum dot (**C**).

In order to achieve the successful design of nanocarriers for natural products, various issues such as optimizing loading capacity, improving biocompatibility, eliminating toxicity, and controlling release needed to be resolved.

8. Summary

Even though the marked increase of life expectancy in recent years can be considered one of our society's greatest achievements, unhealthy eating and lifestyle habits can cause a concomitant dramatic rise of chronic and neurodegenerative diseases. In an effort to reduce the prevalence of oxidative stress associated such chronic diseases, various strategies including consuming multivitamins and antioxidant supplementation have been utilized. Unfortunately, supplementation with high doses of single compounds such as vitamin E failed to show any beneficial effect against chronic diseases and even had harmful effects such as an increased risk of mortality. Unlike well-known antioxidants such as vitamin E, bioactives in natural products that can effectively sequester cytotoxic reactive carbonyl species can provide more targeted action against oxidative stress associated pathologic conditions. Thanks to the recent development of new techniques utilizing high resolution mass spectrometry, reactive carbonyl species sequestering actions of natural products have begun to be identified. In addition, nanotechnologies including nanoparticles and nanocarriers are being explored in order to overcome the limitation of bioavailability and bioefficacy of natural products in humans.

Acknowledgments: Supported by Rural Development Administration, Republic of Korea (PJ011885).

Author Contributions: Sung Won Hwang and Kyung-Jin Yeum proposed the study and designed the manuscript. Sung Won Hwang wrote sections related to nano-techniques and prepared Figures, Kyung-Jin Yeum and Giancarlo Aldini wrote the sections related to reactive carbonyl species, and Yoon-Mi Lee prepared contents of Tables of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gate, L.; Paul, J.; Ba, G.N.; Tew, K.D.; Tapiero, H. Oxidative stress induced in pathologies: The role of antioxidants. *Biomed. Pharmacother.* **1999**, *53*, 169–180. [CrossRef]
2. Hensley, K.; Floyd, R.A. Reactive oxygen species and protein oxidation in aging: A look back, a look ahead. *Arch. Biochem. Biophys.* **2002**, *397*, 377–383. [CrossRef] [PubMed]
3. Ceconi, C.; Boraso, A.; Cargnoni, A.; Ferrari, R. Oxidative stress in cardiovascular disease: Myth or fact? *Arch. Biochem. Biophys.* **2003**, *420*, 217–221. [CrossRef] [PubMed]
4. Carini, M.; Aldini, G.; Facino, R.M. Mass spectrometry for detection of 4-hydroxy-trans-2-nonenal (HNE) adducts with peptides and proteins. *Mass Spectrom. Rev.* **2004**, *23*, 281–305. [CrossRef] [PubMed]

5. Pedchenko, V.K.; Chetyrkin, S.V.; Chuang, P.; Ham, A.J.; Saleem, M.A.; Mathieson, P.W.; Hudson, B.G.; Voziyan, P.A. Mechanism of perturbation of integrin-mediated cell-matrix interactions by reactive carbonyl compounds and its implication for pathogenesis of diabetic nephropathy. *Diabetes* **2005**, *54*, 2952–2960. [CrossRef] [PubMed]
6. Pennathur, S.; Heinecke, J.W. Oxidative stress and endothelial dysfunction in vascular disease. *Curr. Diabetes Rep.* **2007**, *7*, 257–264. [CrossRef]
7. Stanyer, L.; Betteridge, D.J.; Smith, C.C. An investigation into the mechanisms mediating plasma lipoprotein-potentiated beta-amyloid fibrillogenesis. *FEBS Lett.* **2002**, *518*, 72–78. [CrossRef]
8. Uchida, K.; Shiraishi, M.; Naito, Y.; Torii, Y.; Nakamura, Y.; Osawa, T. Activation of stress signaling pathways by the end product of lipid peroxidation. 4-hydroxy-2-nonenal is a potential inducer of intracellular peroxide production. *J. Biol. Chem.* **1999**, *274*, 2234–2242. [CrossRef] [PubMed]
9. Basta, G.; Schmidt, A.M.; De Caterina, R. Advanced glycation end products and vascular inflammation: Implications for accelerated atherosclerosis in diabetes. *Cardiovasc. Res.* **2004**, *63*, 582–592. [CrossRef] [PubMed]
10. Dalle-Donne, I.; Aldini, G.; Carini, M.; Colombo, R.; Rossi, R.; Milzani, A. Protein carbonylation, cellular dysfunction, and disease progression. *J. Cell. Mol. Med.* **2006**, *10*, 389–406. [CrossRef] [PubMed]
11. Harcourt, B.E.; Sourris, K.C.; Coughlan, M.T.; Walker, K.Z.; Dougherty, S.L.; Andrikopoulos, S.; Morley, A.L.; Thallas-Bonke, V.; Chand, V.; Penfold, S.A.; *et al*. Targeted reduction of advanced glycation improves renal function in obesity. *Kidney Int.* **2011**, *80*, 190–198. [CrossRef] [PubMed]
12. Aldini, G.; Vistoli, G.; Regazzoni, L.; Gamberoni, L.; Facino, R.M.; Yamaguchi, S.; Uchida, K.; Carini, M. Albumin is the Main Nucleophilic Target of Human Plasma: A Protective Role Against Pro-atherogenic Electrophilic Reactive Carbonyl Species? *Chem. Res. Toxicol.* **2008**, *21*, 824–835. [CrossRef] [PubMed]
13. Aldini, G.; Regazzoni, L.; Pedretti, A.; Carini, M.; Cho, S.M.; Park, K.M.; Yeum, K.J. An integrated high resolution mass spectrometric and informatics approach for the rapid identification of phenolics in plant extract. *J. Chromatogr. A* **2011**, *1218*, 2856–2864. [CrossRef] [PubMed]
14. Vistoli, G.; De Maddis, D.; Cipak, A.; Zarkovic, N.; Carini, M.; Aldini, G. Advanced glycoxidation and lipoxidation end products (AGEs and ALEs): An overview of their mechanisms of formation. *Free Radic. Res.* **2013**, *47*, 3–27. [CrossRef] [PubMed]
15. Esterbauer, H.; Schaur, R.J.; Zollner, H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* **1991**, *11*, 81–128. [CrossRef]
16. Singh, D.K.; Winocour, P.; Farrington, K. Oxidative stress in early diabetic nephropathy: Fueling the fire. *Nat. Rev. Endocrinol.* **2011**, *7*, 176–184. [CrossRef] [PubMed]
17. Bierhaus, A.; Nawroth, P.P. Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications. *Diabetologia* **2009**, *52*, 2251–2263. [CrossRef] [PubMed]
18. Coughlan, M.T.; Thorburn, D.R.; Penfold, S.A.; Laskowski, A.; Harcourt, B.E.; Sourris, K.C.; Tan, A.L.; Fukami, K.; Thallas-Bonke, V.; Nawroth, P.P.; *et al*. RAGE-induced cytosolic ROS promote mitochondrial superoxide generation in diabetes. *J. Am. Soc. Nephrol.* **2009**, *20*, 742–752. [CrossRef] [PubMed]
19. Beisswenger, P.J.; Howell, S.K.; Touchette, A.D.; Lal, S.; Szwergold, B.S. Metformin reduces systemic methylglyoxal levels in type 2 diabetes. *Diabetes* **1999**, *48*, 198–202. [CrossRef] [PubMed]
20. Rabbani, N.; Thornalley, P.J. The critical role of methylglyoxal and glyoxalase 1 in diabetic nephropathy. *Diabetes* **2014**, *63*, 50–52. [CrossRef] [PubMed]
21. Wlodek, P.; Marcykiewicz, B.; Iciek, M.; Suliga, M.; Smolenski, O.; Kowalczyk-Pachel, D. Thiol levels, protein carbonylation and anaerobic sulfur metabolism in erythrocytes of peritoneal dialysis and predialysis patients. *Nephrology (Carlton)* **2010**, *15*, 755–761. [CrossRef] [PubMed]
22. Shao, C.H.; Capek, H.L.; Patel, K.P.; Wang, M.; Tang, K.; DeSouza, C.; Nagai, R.; Mayhan, W.; Periasamy, M.; Bidasee, K.R. Carbonylation contributes to SERCA2a activity loss and diastolic dysfunction in a rat model of type 1 diabetes. *Diabetes* **2011**, *60*, 947–959. [CrossRef] [PubMed]
23. Uchida, K. Role of reactive aldehyde in cardiovascular diseases. *Free Radic. Biol. Med.* **2000**, *28*, 1685–1696. [CrossRef]
24. Pillon, N.J.; Croze, M.L.; Vella, R.E.; Soulere, L.; Lagarde, M.; Soulage, C.O. The lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE) induces insulin resistance in skeletal muscle through both carbonyl and oxidative stress. *Endocrinology* **2012**, *153*, 2099–2111. [CrossRef] [PubMed]

25. Koh, G.; Lee, D.H.; Woo, J.T. 2-Deoxy-D-ribose induces cellular damage by increasing oxidative stress and protein glycation in a pancreatic beta-cell line. *Metabolism* **2010**, *59*, 325–332. [CrossRef] [PubMed]
26. Ishibashi, Y.; Nishino, Y.; Matsui, T.; Takeuchi, M.; Yamagishi, S. Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. *Metabolism* **2011**, *60*, 1271–1277. [CrossRef] [PubMed]
27. Aldini, G.; Orioli, M.; Rossoni, G.; Savi, F.; Braidotti, P.; Vistoli, G.; Yeum, K.J.; Negrisoli, G.; Carini, M. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. *J. Cell. Mol. Med.* **2011**, *15*, 1339–1354. [CrossRef] [PubMed]
28. Menini, S.; Iacobini, C.; Ricci, C.; Scipioni, A.; Blasetti Fantauzzi, C.; Giaccari, A.; Salomone, E.; Canevotti, R.; Lapolla, A.; Orioli, M.; *et al.* D-Carnosine octylester attenuates atherosclerosis and renal disease in ApoE null mice fed a Western diet through reduction of carbonyl stress and inflammation. *Br. J. Pharmacol.* **2012**, *166*, 1344–1356. [CrossRef] [PubMed]
29. Rahbar, S. Novel inhibitors of glycation and AGE formation. *Cell Biochem. Biophys.* **2007**, *48*, 147–157. [CrossRef] [PubMed]
30. Gasparotto, J.; Somensi, N.; Bortolin, R.C.; Girardi, C.S.; Kunzler, A.; Rabelo, T.K.; Schnorr, C.E.; Moresco, K.S.; Bassani, V.L.; Yatsu, F.K.; *et al.* Preventive supplementation with fresh and preserved peach attenuates CCl4-induced oxidative stress, inflammation and tissue damage. *J. Nutr. Biochem.* **2014**, *25*, 1282–1295. [CrossRef] [PubMed]
31. Sil, R.; Ray, D.; Chakraborti, A.S. Glycyrrhizin ameliorates metabolic syndrome-induced liver damage in experimental rat model. *Mol. Cell. Biochem.* **2015**, *409*, 177–189. [CrossRef] [PubMed]
32. Chen, X.; Mori, T.; Guo, Q.; Hu, C.; Ohsaki, Y.; Yoneki, Y.; Zhu, W.; Jiang, Y.; Endo, S.; Nakayama, K.; *et al.* Carbonyl stress induces hypertension and cardio-renal vascular injury in Dahl salt-sensitive rats. *Hypertens. Res.* **2013**, *36*, 361–367. [CrossRef] [PubMed]
33. Cuccurullo, C.; Iezzi, A.; Fazia, M.L.; De Cesare, D.; Di Francesco, A.; Muraro, R.; Bei, R.; Ucchino, S.; Spigonardo, F.; Chiarelli, F.; *et al.* Suppression of RAGE as a basis of simvastatin-dependent plaque stabilization in type 2 diabetes. *Arterioscler. Thromb. Vasc. Biol.* **2006**, *26*, 2716–2723. [CrossRef] [PubMed]
34. Uchida, K.; Toyokuni, S.; Nishikawa, K.; Kawakishi, S.; Oda, H.; Hiai, H.; Stadtman, E.R. Michael addition-type 4-hydroxy-2-nonenal adducts in modified low-density lipoproteins: Markers for atherosclerosis. *Biochemistry* **1994**, *33*, 12487–12494. [CrossRef] [PubMed]
35. Lee, S.J.; Lee, K.W. Protective effect of (−)-epigallocatechin gallate against advanced glycation endproducts-induced injury in neuronal cells. *Biol. Pharm. Bull.* **2007**, *30*, 1369–1373. [CrossRef] [PubMed]
36. Meng, X.; Sun, G.; Ye, J.; Xu, H.; Wang, H.; Sun, X. Notoginsenoside R1-mediated neuroprotection involves estrogen receptor-dependent crosstalk between Akt and ERK1/2 pathways: A novel mechanism of Nrf2/ARE signaling activation. *Free Radic. Res.* **2014**, *48*, 445–460. [CrossRef] [PubMed]
37. Lu, J.; Wu, D.M.; Zheng, Y.L.; Hu, B.; Zhang, Z.F.; Ye, Q.; Liu, C.M.; Shan, Q.; Wang, Y.J. Ursolic acid attenuates D-galactose-induced inflammatory response in mouse prefrontal cortex through inhibiting AGEs/RAGE/NF-kappaB pathway activation. *Cereb. Cortex* **2010**, *20*, 2540–2548. [CrossRef] [PubMed]
38. Manda, K.; Ueno, M.; Anzai, K. Melatonin mitigates oxidative damage and apoptosis in mouse cerebellum in'duced by high-LET 56Fe particle irradiation. *J. Pineal Res.* **2008**, *44*, 189–196. [CrossRef] [PubMed]
39. Lu, J.; Wu, D.M.; Zheng, Z.H.; Zheng, Y.L.; Hu, B.; Zhang, Z.F. Troxerutin protects against high cholesterol-induced cognitive deficits in mice. *Brain* **2011**, *134*, 783–797. [CrossRef] [PubMed]
40. Farr, S.A.; Ripley, J.L.; Sultana, R.; Zhang, Z.; Niehoff, M.L.; Platt, T.L.; Murphy, M.P.; Morley, J.E.; Kumar, V.; Butterfield, D.A. Antisense oligonucleotide against GSK-3beta in brain of SAMP8 mice improves learning and memory and decreases oxidative stress: Involvement of transcription factor Nrf2 and implications for Alzheimer disease. *Free Radic. Biol. Med.* **2014**, *67*, 387–395. [CrossRef] [PubMed]
41. Ishrat, T.; Hoda, M.N.; Khan, M.B.; Yousuf, S.; Ahmad, M.; Khan, M.M.; Ahmad, A.; Islam, F. Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimers type (SDAT). *Eur. Neuropsychopharmacol.* **2009**, *19*, 636–647. [CrossRef] [PubMed]

42. Joshi, G.; Perluigi, M.; Sultana, R.; Agrippino, R.; Calabrese, V.; Butterfield, D.A. *In vivo* protection of synaptosomes by ferulic acid ethyl ester (FAEE) from oxidative stress mediated by 2,2-azobis(2-amidino-propane)dihydrochloride (AAPH) or Fe(2+)/H(2)O(2): Insight into mechanisms of neuroprotection and relevance to oxidative stress-related neurodegenerative disorders. *Neurochem. Int.* **2006**, *48*, 318–327. [PubMed]
43. Aluise, C.D.; Miriyala, S.; Noel, T.; Sultana, R.; Jungsuwadee, P.; Taylor, T.J.; Cai, J.; Pierce, W.M.; Vore, M.; Moscow, J.A.; *et al*. 2-Mercaptoethane sulfonate prevents doxorubicin-induced plasma protein oxidation and TNF-alpha release: Implications for the reactive oxygen species-mediated mechanisms of chemobrain. *Free Radic. Biol. Med.* **2011**, *50*, 1630–1638. [CrossRef] [PubMed]
44. Mitchel, R.E.; Birnboim, H.C. The use of Girard-T reagent in a rapid and sensitive methods for measuring glyoxal and certain other alpha-dicarbonyl compounds. *Anal. Biochem.* **1977**, *81*, 47–56. [CrossRef]
45. Vistoli, G.; de Maddis, D.; Straniero, V.; Pedretti, A.; Pallavicini, M.; Valoti, E.; Carini, M.; Testa, B.; Aldini, G. Exploring the space of histidine containing dipeptides in search of novel efficient RCS sequestering agents. *Eur. J. Med. Chem.* **2013**, *66*, 153–160. [CrossRef] [PubMed]
46. Liu, Y.; Xu, G.; Sayre, L.M. Carnosine inhibits (*E*)-4-hydroxy-2-nonenal-induced protein cross-linking: Structural characterization of carnosine-HNE adducts. *Chem. Res. Toxicol.* **2003**, *16*, 1589–1597. [CrossRef] [PubMed]
47. Sasaki, N.A.; Garcia-Alvarez, M.C.; Wang, Q.; Ermolenko, L.; Franck, G.; Nhiri, N.; Martin, M.T.; Audic, N.; Potier, P. N-Terminal 2,3-diaminopropionic acid (Dap) peptides as efficient methylglyoxal scavengers to inhibit advanced glycation endproduct (AGE) formation. *Bioorg. Med. Chem.* **2009**, *17*, 2310–2320. [CrossRef] [PubMed]
48. Colzani, M.; Criscuolo, A.; De Maddis, D.; Garzon, D.; Yeum, K.J.; Vistoli, G.; Carini, M.; Aldini, G. A novel high resolution MS approach for the screening of 4-hydroxy-trans-2-nonenal sequestering agents. *J. Pharm. Biomed. Anal.* **2014**, *91*, 108–118. [CrossRef] [PubMed]
49. Wang, X.; Ouyang, Y.; Liu, J.; Zhu, M.; Zhao, G.; Bao, W.; Hu, F.B. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* **2014**, *349*. [CrossRef] [PubMed]
50. Neuhouser, M.L.; Wassertheil-Smoller, S.; Thomson, C.; Aragaki, A.; Anderson, G.L.; Manson, J.E.; Patterson, R.E.; Rohan, T.E.; van Horn, L.; Shikany, J.M.; *et al*. Multivitamin Use and Risk of Cancer and Cardiovascular Disease in the Women's Health Initiative Cohorts. *Arch. Intern. Med.* **2009**, *169*, 294–304. [CrossRef] [PubMed]
51. Bjelakovic, G.; Nikolova, D.; Gluud, L.L.; Simonetti, R.G.; Gluud, C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst. Rev.* **2008**, *3*. [CrossRef]
52. Miller, E.R., 3rd; Pastor-Barriuso, R.; Dalal, D.; Riemersma, R.A.; Appel, L.J.; Guallar, E. Meta-analysis: High-dosage vitamin E supplementation may increase all-cause mortality. *Ann. Intern. Med.* **2005**, *142*, 37–46. [CrossRef] [PubMed]
53. Lawson, K.A.; Wright, M.E.; Subar, A.; Mouw, T.; Hollenbeck, A.; Schatzkin, A.; Leitzmann, M.F. Multivitamin use and risk of prostate cancer in the National Institutes of Health-AARP Diet and Health Study. *J. Natl. Cancer Inst.* **2007**, *99*, 754–764. [CrossRef] [PubMed]
54. Aldini, G.; Carini, M.; Beretta, G.; Bradamante, S.; Facino, R.M. Carnosine is a quencher of 4-hydroxy-nonenal: Through what mechanism of reaction? *Biochem. Biophys. Res. Commun.* **2002**, *298*, 699–706. [CrossRef]
55. Aldini, G.; Granata, P.; Orioli, M.; Santaniello, E.; Carini, M. Detoxification of 4-hydroxynonenal (HNE) in keratinocytes: Characterization of conjugated metabolites by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2003**, *38*, 1160–1168. [CrossRef] [PubMed]
56. Janssen, B.; Hohenadel, D.; Brinkkoetter, P.; Peters, V.; Rind, N.; Fischer, C.; Rychlik, I.; Cerna, M.; Romzova, M.; de Heer, E.; *et al*. Carnosine as a protective factor in diabetic nephropathy: Association with a leucine repeat of the carnosinase gene CNDP1. *Diabetes* **2005**, *54*, 2320–2327. [CrossRef] [PubMed]
57. Gallant, S.; Semyonova, M.; Yuneva, M. Carnosine as a potential anti-senescence drug. *Biochemistry* **2000**, *65*, 866–868. [PubMed]
58. Hipkiss, A.R. Glycation, ageing and carnosine: Are carnivorous diets beneficial? *Mech. Ageing Dev.* **2005**, *126*, 1034–1039. [CrossRef] [PubMed]

59. Lee, Y.T.; Hsu, C.C.; Lin, M.H.; Liu, K.S.; Yin, M.C. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur. J. Pharmacol.* **2005**, *513*, 145–150. [CrossRef] [PubMed]
60. Kurata, H.; Fujii, T.; Tsutsui, H.; Katayama, T.; Ohkita, M.; Takaoka, M.; Tsuruoka, N.; Kiso, Y.; Ohno, Y.; Fujisawa, Y.; *et al*. Renoprotective effects of l-carnosine on ischemia/reperfusion-induced renal injury in rats. *J. Pharmacol. Exp. Ther.* **2006**, *319*, 640–647. [CrossRef] [PubMed]
61. Cahill, L.E.; Fontaine-Bisson, B.; El-Sohemy, A. Functional genetic variants of glutathione S-transferase protect against serum ascorbic acid deficiency. *Am. J. Clin. Nutr.* **2009**, *90*, 1411–1417. [CrossRef] [PubMed]
62. Milman, U.; Blum, S.; Shapira, C.; Aronson, D.; Miller-Lotan, R.; Anbinder, Y.; Alshiek, J.; Bennett, L.; Kostenko, M.; Landau, M.; *et al*. Vitamin E supplementation reduces cardiovascular events in a subgroup of middle-aged individuals with both type 2 diabetes mellitus and the haptoglobin 2–2 genotype: A prospective double-blinded clinical trial. *Arterioscler. Thromb. Vasc. Biol.* **2008**, *28*, 341–347. [CrossRef] [PubMed]
63. Freedman, B.I.; Hicks, P.J.; Sale, M.M.; Pierson, E.D.; Langefeld, C.D.; Rich, S.S.; Xu, J.; McDonough, C.; Janssen, B.; Yard, B.A.; van der Woude, F.J.; Bowden, D.W. A leucine repeat in the carnosinase gene CNDP1 is associated with diabetic end-stage renal disease in European Americans. *Nephrol. Dial. Transplant.* **2007**, *22*, 1131–1135. [CrossRef] [PubMed]
64. Lee, Y.M.; Han, S.I.; Won, Y.J.; Lee, E.; Park, E.; Hwang, S.Y.; Yeum, K.J. Black Rice with Giant Embryo Attenuates Obesity-Associated Metabolic Disorders in *ob/ob* Mice. *J. Agric. Food Chem.* **2015**. [CrossRef] [PubMed]
65. Wang, S.; Su, R.; Nie, S.; Sun, M.; Zhang, J.; Wu, D.; Moustaid-Moussa, N. Application of nanotechnology in improving bioavailability and bioactivity of diet-derived phytochemicals. *J. Nutr. Biochem.* **2014**, *25*, 363–376. [CrossRef] [PubMed]
66. Gunasekaran, T.; Haile, T.; Nigusse, T.; Dhanaraju, M.D. Nanotechnology: An effective tool for enhancing bioavailability and bioactivity of phytomedicine. *Asian Pac. J. Trop. Biomed.* **2014**, *4*, S1–S7. [CrossRef] [PubMed]
67. Shakeri, A.; Sahebkar, A. Nanotechnology: A Successful Approach to Improve Oral Bioavailability of Phytochemicals. *Recent Pat. Drug Deliv. Formul.* **2015**. [CrossRef]
68. De Pace, R.C.; Liu, X.; Sun, M.; Nie, S.; Zhang, J.; Cai, Q.; Gao, W.; Pan, X.; Fan, Z.; Wang, S. Anticancer activities of (−)-epigallocatechin-3-gallate encapsulated nanoliposomes in MCF7 breast cancer cells. *J. Liposome Res.* **2013**, *23*, 187–196. [CrossRef] [PubMed]
69. Hu, B.; Ting, Y.; Zeng, X.; Huang, Q. Bioactive peptides/chitosan nanoparticles enhance cellular antioxidant activity of (−)-epigallocatechin-3-gallate. *J. Agric. Food Chem.* **2013**, *61*, 875–881. [CrossRef] [PubMed]
70. Tan, B.J.; Liu, Y.; Chang, K.L.; Lim, B.K.; Chiu, G.N. Perorally active nanomicellar formulation of quercetin in the treatment of lung cancer. *Int. J. Nanomed.* **2012**, *7*, 651–661. [PubMed]
71. Li, H.; Zhao, X.; Ma, Y.; Zhai, G.; Li, L.; Lou, H. Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. *J. Control Release* **2009**, *133*, 238–244. [CrossRef] [PubMed]
72. Li, W.; Yi, S.; Wang, Z.; Chen, S.; Xin, S.; Xie, J.; Zhao, C. Self-nanoemulsifying drug delivery system of persimmon leaf extract: Optimization and bioavailability studies. *Int. J. Pharm.* **2011**, *420*, 161–171. [CrossRef] [PubMed]
73. Xie, X.; Tao, Q.; Zou, Y.; Zhang, F.; Guo, M.; Wang, Y.; Wang, H.; Zhou, Q.; Yu, S. PLGA nanoparticles improve the oral bioavailability of curcumin in rats: Characterizations and mechanisms. *J. Agric. Food Chem.* **2011**, *59*, 9280–9289. [CrossRef] [PubMed]
74. Yallapu, M.M.; Gupta, B.K.; Jaggi, M.; Chauhan, S.C. Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. *J. Colloid Interface Sci.* **2010**, *351*, 19–29. [CrossRef] [PubMed]
75. Liu, Z.; Robinson, J.T.; Sun, X.; Dai, H. PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. *J. Am. Chem. Soc.* **2008**, *130*, 10876–10877. [CrossRef] [PubMed]
76. Sandhir, R.; Yadav, A.; Sunkaria, A.; Singhal, N. Nano-antioxidants: An emerging strategy for intervention against neurodegenerative conditions. *Neurochem. Int.* **2015**, *89*, 209–226. [CrossRef] [PubMed]
77. Novoselov, K.S.; Geim, A.K.; Morozov, S.V.; Jiang, D.; Zhang, Y.; Dubonos, S.V.; Grigorieva, I.V.; Firsov, A.A. Electric field effect in atomically thin carbon films. *Science* **2004**, *306*, 666–669. [CrossRef] [PubMed]
78. Dreyer, D.R.; Park, S.; Bielawski, C.W.; Ruoff, R.S. The chemistry of graphene oxide. *Chem. Soc. Rev.* **2010**, *39*, 228–240. [CrossRef] [PubMed]

79. Shao, Y.; Wang, J.; Wu, H.; Liu, J.; Aksay, I.A.; Lina, Y. Graphene based elecrochemical sensors and biosensors: A review. *Electroanalysis* **2010**, *22*, 1027–1036. [CrossRef]
80. Orive, G.; Anitua, E.; Pedraz, J.L.; Emerich, D.F. Biomaterials for promoting brain protection, repair and regeneration. *Nat. Rev. Neurosci.* **2009**, *10*, 682–692. [CrossRef] [PubMed]
81. Lu, C.H.; Yang, H.H.; Zhu, C.L.; Chen, X.; Chen, G.N. A graphene platform for sensing biomolecules. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 4785–4787. [CrossRef] [PubMed]

Sample Availability: Not available.