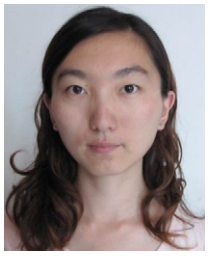
editorial

Xue-Juan Li



Na Gao

Hong-Yu Zhang*

Natural inspirations for antioxidant drug discovery

As reactive oxidative species (ROS) are involved in the pathogenesis of many diseases, finding efficient antioxidants as therapeutic agents has been one of the hottest areas in biomedicine. By searching PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez?db=PubMed), it was found that more than 300,000 papers associated with antioxidants were published from 1980 to 2008 and the amount of the publications increased steadily (Fig. 1). However, only relatively trivial success has been attained in antioxidant-based drug discovery over this period. According to the MDL Drug Data Report (MDDR) database, only two antioxidant drugs have been launched (i.e. idebenone and edaravone) in the past 30 years. Thus, it seems that it is urgent to improve the translational efficiency of antioxidant research. Considering the fact that, evolutionarily, organisms have accumulated a massive experience in combating ROS, it is of great interest to examine how such organisms use antioxidants, which might provide inspiration for future antioxidant drug discovery.

Those diseases that might benefit from antioxidant therapy can be divided into two classes, namely, those that have been induced by acute ROS (e.g. reperfusion injury and inflammation) and those induced by chronic ROS (e.g. atherosclerosis, cancer, dementia and diabetic vascular disease) [1]. Interestingly, the naturally selected antioxidant systems can also be divided into 'strong' and 'common'. The former includes those used by radiation-resistant bacteria, which have to survive acute ROS [2]. The latter includes those used by mitochondria, where ROS is produced chronically [3].

Composition of the strong natural antioxidant system

The most radiation-resistant organism discovered to date is *Deinococcus radiodurans* that was first isolated from canned meat that had received 4000 Gy of ionizing radiation. *D. radiodurans* is able to withstand 50–100 times more ionizing radiation than *Escherichia coli* and can survive an acute ionizing radiation dose of 1.5 Mrad with no obvious loss of viability [4]. The unique cellular structure and ringlike genome, high-efficiency DNA damage repair system and remarkable antioxidative capacity are considered to be responsible for the unusually high radiation resistance of *D. radiodurans* [2].

The sophisticated antioxidant system of *D. radiodurans* not only consists of antioxidant enzymes, for example, Mn- or Fe-superoxide dismutase, Cu,Zn-superoxide dismutase, catalase, peroxidase, thiol-alkyl hydroperoxide reductases, thioredoxin reductase/alkyl hydroperoxide reductase, peptide methionine sulfoxide reductase and glutaredoxin [5], but also comprises small-molecule

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2009.08.003

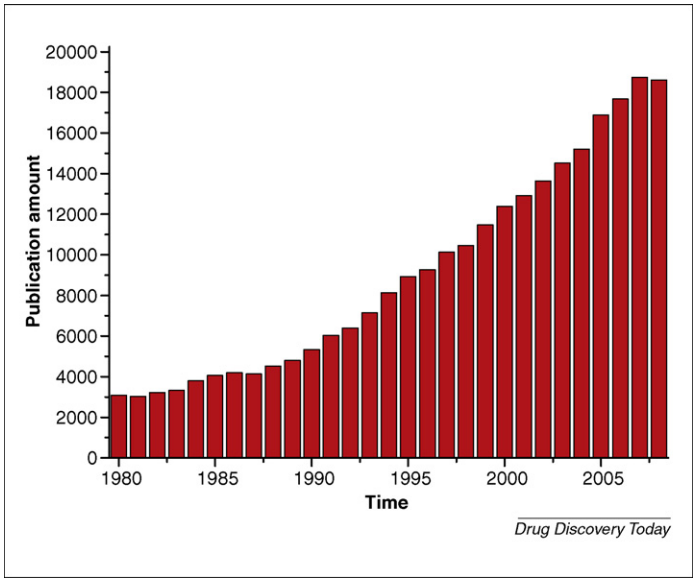


FIGURE 1

Publications associated with antioxidants increased steadily in the past three decades.

agents, such as nonenzymic Mn(II) complexes, lipoic acid, carotenoids (beta-carotene, lycopene and phytoene) and folates [2,5]. The latter chemical antioxidants have been reported to provide immediate cytosolic protection for *D. radiodurans* from ionizing radiation-induced ROS [2,5].

Composition of the common natural antioxidant system

Mitochondria are considered to be the richest source of ROS encountered in the normal life of eukaryotes. ROS can attack almost all biomolecules present in their vicinity and cause oxidative stress in cells. To prevent ROS-mediated damage, mitochondria have become equipped, through biological evolution, with a multilevel ROS defense system [3], consisting of enzymatic and nonenzymatic components. The former includes Mn-superoxide dismutase, catalase, glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase, glutathione reductase, peroxiredoxins, glutaredoxin, thioredoxin and thioredoxin reductase [3]. The latter includes α-tocopherol, ascorbic acid, ubiquinol, lipoic acid, folates, cytochrome *c*, reduced glutathione and nicotinamide adenine dinucleotide phosphate, which prevent mitochondria from oxidative damage through removing ROS [3,6,7].

Phenolic antioxidants are rarely used by nature

Current studies of antioxidant chemistry and biology have highlighted phenolic antioxidants as a particularly attractive area in which to focus. A large number of naturally occurring phenols, especially catechols, have been shown to possess potent *in vitro* antioxidant activity [8]. However, phenolic antioxidants are not prevalent in natural antioxidant systems, an assertion corroborated by a comprehensive examination of the metabolic composition of *D. radiodurans* and mitochondria.

To establish the metabolome of *D. radiodurans*, the genome was reannotated, allowing identification of the metabolic enzymes. Then, the metabolites associated with the enzymes were collected according to the reaction pathway information provided by KEGG. This procedure results in a metabolome of 287 metabolic products. It is interesting to note that only five phenols (i.e. aryl alcohol, 4-hydroxybenzoate, 4-hydroxyphenylpyruvate, naringenin chalcone, protein tyrosine) are involved in this metabolome. According to the structure–activity relationships of phenolic antioxidants [9], none of the phenols is efficient in scavenging ROS, because they have no electron-donating substituents at the *para* or *ortho* positions of the hydroxyl group.

The metabolome of mitochondria has been collected by Duarte *et al.* [10], and comprises 389 metabolites (including 14 phenols). Of these phenols, only ubiquinol and a catechol (i.e. 3-decaprenyl-4,5-dihdydroxybenzoate) are potential antioxidants (α-tocopherol is not included in this metabolome). Taken together, it seems that in both strong and common natural antioxidant systems, phenolic compounds are scarce, which has important implications for addressing some interesting issues in antioxidant chemistry and biology, for example, why the *in vivo* efficacy of phenolic antioxidants is poor.

Despite the strong *in vitro* antioxidant activity of various phenols [8], they are trivial radical scavengers in *in vivo* systems [11]. In fact, although some FDA-approved drugs are phenolic compounds (even catechols), none of them is annotated as an antioxidant [12]. In addition, extracts of green tea extracts containing polyphenols (Veregen), have been approved by FDA [13] and are indicated for topical treatment of external genital and perianal warts, properties unrelated to the neutralization of ROS. The contrast of the *in vitro* and *in vivo* antioxidant effects of phenols had previously been explained in terms of the poor bioavailability of exogenous phenolic compounds [14]. However, the present analysis indicates that even phenolic antioxidants are excluded from the endogenous ROS defense systems, which implies that we should go beyond bioavailability to explain the dichotomy of phenolic antioxidants. A possible mechanism underlying this phenomenon is that, in biological systems, the phenolic hydroxyl groups tend to form hydrogen bonds with surrounding polar molecules (including water), which could hinder the hydrogen donation of phenols and thus mask their radical-scavenging capacity [15]. This explanation is supported by the fact that α-tocopherol and ubiquinol, the only two phenolic antioxidants employed by the natural antioxidant systems, are localized in membranes, where polar solvents are largely absent.

Implications for antioxidant drug discovery

It is intriguing to note that some of the small-molecule antioxidants used by *D. radiodurans* and mitochondria have been developed as drugs or recognized as promising drug candidates. For instance, lipoic acid was launched as an antioxidant drug 50 years ago (according to the MDDR database); the antioxidant drug idebenone was approved 20 years ago and is a ubiquinol-like compound; and Mn(II)-containing catalytic antioxidants have shown efficacy in several oxidative stress models of human disease, such as cardiovascular, neurodegenerative and inflammatory lung disorders [16]. Thus, it seems that the naturally selected antioxidant systems could provide inspirations for finding antioxidant drugs. First, we suggest that to find antioxidant drugs to treat acute ROS-related diseases, a combination of Mn(II)-containing catalytic antioxidants and lipoic acid, carotenoids and folates is a promising candidate. Second, we believe that, in a broad sense, phenols are

not good starting points for finding antioxidant drugs, except to prevent chronic ROS-mediated damage to membranes. As a result, antioxidant drug discovery would better turn to nonphenols, albeit phenols indeed show strong antioxidant potential in *in vitro* systems and could find important use in chemical and food industries.

Conclusion

To survive acute or chronic ROS attack, organisms have evolved efficient antioxidant systems, which consist of enzymes and small-molecule agents. The latter has important implications for antioxidant drug discovery. However, it should be pointed out that these endogenous antioxidants exert their effects mainly through chain-breaking mechanisms, thus the present conclusion is only relevant to finding drugs capable of preventing or inhibiting this process. Because other pathways exist to neutralize ROS, such as inhibiting ROS-producing enzymes and chelating transition metals, space for antioxidant drug discovery is, in fact, still ample.

References

1 Maxwell, S.R.J. (1997) Anti-oxidant therapy: does it have a role in the treatment of human disease? *Exp. Opin. Invest. Drugs* 6, 211–236

2 Daly, M.J. (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nat. Rev. Microbiol.* 7, 237–245

3 Andreyev, A.Y. *et al.* (2005) Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc.)* 70, 200–214

4 Venkateswaran, A. *et al.* (2000) Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl. Environ. Microbiol.* 66, 2620–2626

5 Zhang, H.-Y. *et al.* (2009) Antioxidants used by *Deinococcus radiodurans* and implications for antioxidant drug discovery. *Nat. Rev. Microbiol.* 7 10.1038/nrmicro2073-c1

6 Liu, J. and Ames, B.N. (2005) Reducing mitochondrial decay with mitochondrial nutrients to delay and treat cognitive dysfunction, Alzheimer's disease, and Parkinson's disease. *Nutr. Neurosci.* 8, 67–89

7 Chang, C.M. *et al.* (2007) Folate deprivation promotes mitochondrial oxidative decay: DNA large deletions, cytochrome *c* oxidase dysfunction, membrane depolarization and superoxide overproduction in rat liver. *Br. J. Nutr.* 97, 855–863

8 Rice-Evans, C.A. *et al.* (1996) Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.* 20, 933–956

9 Zhang, H.-Y. (2005) Structure–activity relationships and rational design strategies for radical-scavenging antioxidants. *Curr. Comput.-Aided Drug Design* 1, 257–273

10 Duarte, N.C. *et al.* (2007) Global reconstruction of the human metabolic network based on genomic and bibliomic data. *Proc. Natl. Acad. Sci. U. S. A.* 104, 1777–1782

11 Azzi, A. *et al.* (2004) Free radical biology: terminology and critical thinking. *FEBS Lett.* 558, 3–6

12 Yang, D.P. *et al.* (2007) How many drugs are catecholics. *Molecules* 12, 878–884

13 Schmidt, B.M. *et al.* (2007) Revisiting the ancient concept of botanical therapeutics. *Nat. Chem. Biol.* 3, 360–366

14 Shen, L. *et al.* (2007) How to understand the dichotomy of antioxidants. *Biochem. Biophys. Res. Commun.* 362, 543–545

15 Halliwell, B. *et al.* (2005) Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? antioxidant or not?. *Am. J. Clin. Nutr.* 81, 268S–276S

16 Day, B.J. (2004) Catalytic antioxidants: a radical approach to new therapeutics. *Drug Discov. Today* 9, 557–566

Xue-Juan Li, Na Gao

Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Center for Advanced Study, Shandong University of Technology, Zibo 255049, PR China

Hong-Yu Zhang*

Institute of Bioinformatics and State Key Laboratory of Crop Genetic Improvement, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

e-mail: zhy630@mail.hzau.edu.cn