Edaravone: Potential target for cardioprotection and neuroprotection

Viraj H. Mankar*,¶,‡,

¶ Lovely Professional University, Phagwara 144411, Punjab, India

Abstract:

Edaravone is a potentially useful cardio and neuro-protective agent, as it has been used for the therapy of cerebral infarction and acute ischemic stroke. In order to prevent the injuries caused by ischemic stroke and generated reactive oxygen species will be scavenged by antioxidant mechanism of edaravone. However, some of edaravone derivatives are also exhibit highest anticancer, antimicrobial, antibacterial, anti-inflammatory, antitumor, antidepressant activity. Because of these tremendous applications of pyrazoline backbone structure, new derivatives of Edaravone are being synthesized to enhance the property of the drug. Furthermore, additional clinical evaluations are also required to verify the efficacy of these chemical moieties in cardio and neuroprotection.

INTRODUCTION

Cardiovascular diseases (CVD) are a leading cause of death in world. These diseases contribute to about 50% of global mortality.[1] According to the World Health Organisation and the World Bank, in India, deaths with CVD have increased proportionally with the expanding population. The term cardiovascular disease involves a several diseases including, ischemic heart disease (IHD), cerebrovascular disease and other related cardiovascular diseases, such as myocardial infarction (MI). Cardiovascular diseases affect the various components of the cardiovascular system like arteries and the myocardium. Diseases including CVD, cancers, diabetes, and chronic obstructive pulmonary disease are affected by common lifestyle habits such as diet, physical activity, and tobacco consumption.[2]

Cardiovascular diseases arise basically because of impaired blood flow through the arteries and blood vessels. This manifests in the form of atherosclerosis which is the most common cause of cardiovascular diseases. Atherosclerosis is a pathological process wherein intima and media of arterial vessels undergo structural changes.[3] This happens mainly because of cholesterol accumulation, inflammatory cell infiltration and vascular smooth muscle cell migration.[3]Atherosclerosis therefore involves the deposition of fatty deposits (atheroma) in blood vessels.

However, it is the damage of the endothelium by reactive oxygen species (ROS) which causes formation of atheroma. Several degenerative diseases are now believed to be caused, or exacerbated, by ROS.[4] Numerous *in vitro* and animal studies have shown that oxidative modification of the low density lipoproteins in our body is the main initial event in atherosclerosis. This oxidation proceeds by multiple pathways and is mediated by free radicals.[5]

Free radical is an atom or molecule with one or more unpaired electrons. In cells, most free radical damage is because of ROS. These includes, molecular oxygen(O_2), hydroxide radical(·OH), superoxide radical(·OOR), singlet oxygen(O), hydrogen peroxide(H_2O_2), lipid peroxide etc.

In biological system, free radicals are produced by and arachi-donate, xanthine oxidase, mitochondria, peroxisomes, phagocytic pathways, ischemia/reperfusion injury along with exercise. However, externally free radicals are produced by cigarette smoke, other environmental pollutants, UV radiation, ozone, certain drugs, pesticides, industrial solvents.[6]

Reduced blood supply to the brain or heart tissues called Ischaemia, which is a symptom of stroke and also of myocardial infarction.[7] Acute Ischemic Stroke (AIS) affects approximately 80% of population than all other strokes, which seriously harming to human health. Several major and uncommon complications in the body which lead to an acute heterogeneous syndrome known as an ischemic stroke, which result in blockage of blood vessels, that supply blood oxygen to brain tissues. In brain, after deprivation of oxygen to cells, some neurons will die within minutes along with further immediate irreversible brain injury. This will lead to stop brain function, partially or completely.[8] Reactive radical species is the potential target for therapeutic development in the treatment of Acute Ischemic Stroke, due to the free radical induced oxidative stress is a leading complications in the ischemic stroke event, which will be activate after vascular blockage.[9]

The degenerative diseases are a result of uncontrolled free radical reactions. The Oxygen Free Radical (OFR) related mutagenesis causes cancer initiation and progression.[10] Almost all cardiovascular diseases begins due to the cell damage caused by free radicals in biological systems. Free-radical mechanisms have been used in the pathophysiology of several human diseases, including atherosclerosis cancer, malaria, and rheumatoid arthritis along with several neurological disorders.[11]

Body has its own natural defense mechanism; it scavenges these free radicals with help of substances known as antioxidants. As the name suggests, they work against oxidative damage by neutralizing the harmful radicals. Antioxidants are any substance which at low concentrations, significantly delays or inhibits oxidation of a substrate.[12]

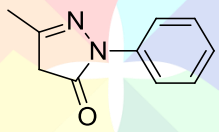
Antioxidant defense mechanism classified into three main types:[13]

a. The primary defence of an antioxidants which includes enzymes such as glutathione reductase (GR), superoxide's dismutase (SOD), catalases (CAT), glutathione reductases (GR) and some minerals such as Cu, Zn, Se, etc.

b. The secondary defence antioxidants which includes, albumin, carotenoids, glutathione (GSH), flavonoids, vitamin C and E, etc.

c. The tertiary defence of antioxidants which includes, a complex set of enzymes for regeneration of damaged proteins, damaged DNA, oxidized lipids species, Such as, lipase, protease, DNA repairs enzymes, including methionine sulphoxide reductase, transferases, etc. Antioxidants scavenges the effect of reactive oxygen free radicals and thus help in preventing diseases.[13]

Edaravone (1) or 1-phenyl-3-Methyl--1H-pyrazol-5-one is a potent free radical (•) scavenger, and is also used in clinical treatment in Japan with chronic brain infarction patients. This pyrazoline moiety also has activity against myocardial injury followed by ischemia/ reperfusion injury with patients having acute myocardial infarction.[17]



1

Figure 1: Structure of Edaravone

In japan since 2001, Edaravone has been used in treatment acute ischemic stroke as it is originally has a potent free radical scavenging activity .[16] These activities of Edaravone includes, blockage of lipoxygenase enzyme metabolic of aracchidonic acid which was using scavenging of hydroxyl radicals, inhibits alloxan-generated lipid peroxidation, and scavenging of active oxygen species along with enhancement of prostacyclin production resulting in protection of different cells, including endothelial cells, shows against damage through oxygen free radicals. Also, it has been reported that edaravone decrease ROS leads to improving endothelial function in chain smokers. Thus, in patients with cardiovascular diseases, it is essential to use selective drug that improves endothelial function.[17]

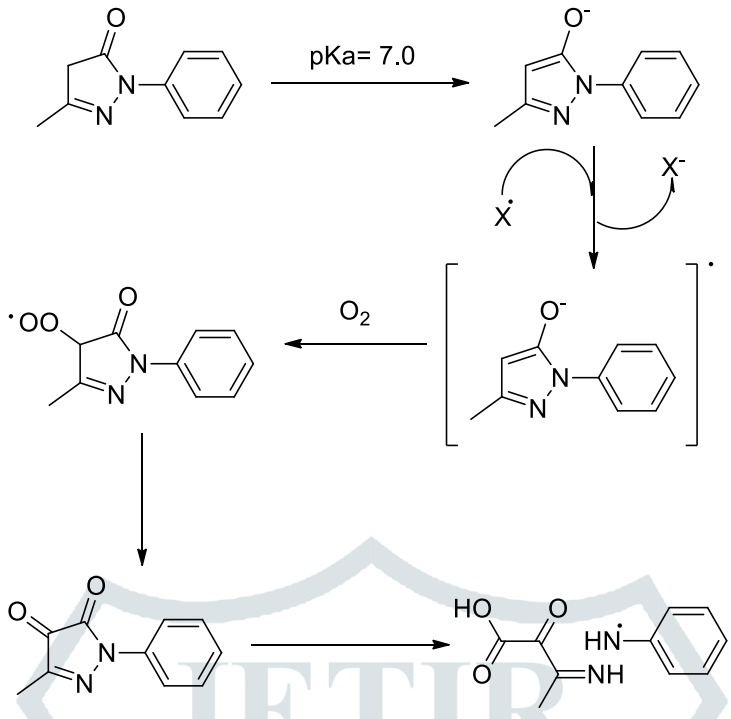


Figure 2: Mechanism of antioxidant action of Edaravone

Antioxidant mechanism of edaravone is responsible for its protection against ischemia and enolate species **(2)** can interact with both hydroxyl radicals (·OH) and peroxyl (LOO·) radicals, after it form a stable oxidation product, 3-(phenylhydrazono)-2-oxo -butanoic acid (OPB) **(3)** through a free radical intermediate species (Figure.3).

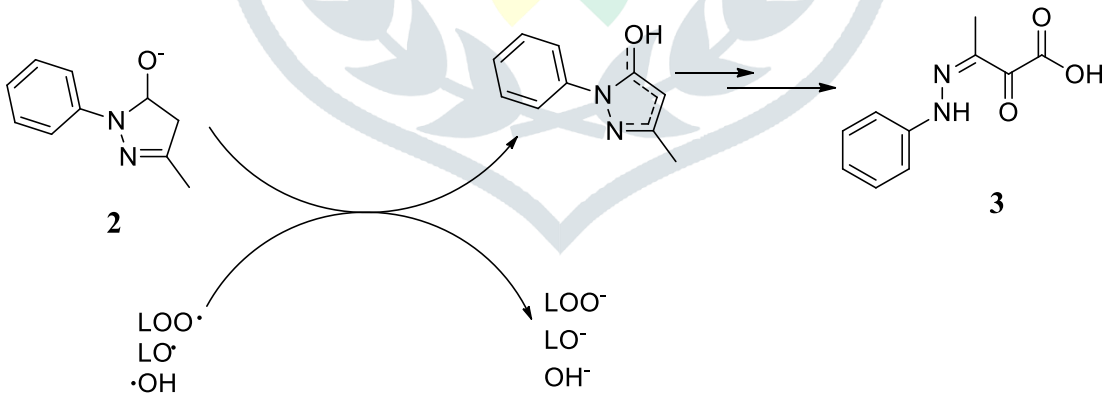
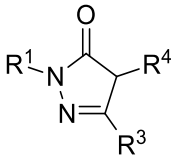


Figure 3: Reaction of enolate form of Edaravone with free radicals

Several edaravone derivatives were synthesized **(4)** and evaluated for their antioxidant activity in hydroxyl radical scavenging activity[18]. It was found pyrazolone derivatives had a much greater ability to quench free radicals than edaravone alone. Antioxidant activity was due to increase of its active anionic form with a hydrogen-bonded intra-molecular base.[18]



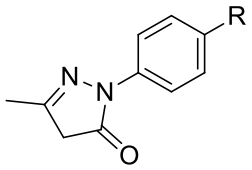
4

Figure 4: Substituent positions in Edaravone

Sr. No.	R^1	R^3	R^4	Oxidation Potentials(mV)
1	4-ClPhenyl-	CH_3–	H	473
2	4-CH_3O-Phenyl–	CH_3–	H	678
3	Phenyl	4 -NO_2-Phenyl	H	419
4	Phenyl–	Isopropenyl–	H	387
5	Phenyl-	CH_3–	Phenyl-	227
6	Phenyl–	CH_3–	Cyclopropyl–	275

Table 1. Substitutions on compound 4

Also, series of polyvalent drugs reported by combining edaravone with NO-donor moieties **(5)** [19]. All compounds exhibits higher antioxidant potency together along with vasodilator properties. These products are useful potential treatment in cardiovascular diseases in which Endothelium Dependent Relaxation Factor (EDRF) deficiency.[19]



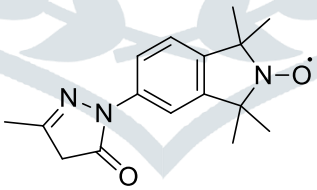
5

Figure 5: Derivatives with NO donor groups

Sr. No.	R
1	$O(CH_2)_3ONO_2$
2	$O(CH_2)_6ONO_2$

Table 2. Substitutions on compound 5

Nitroxides are commonly used as antioxidant in various *in vivo* and *in vitro* applications as it is having stability than normal free radicals. Radical scavenging properties of nitroxides can reduce levels of ROS generated oxidative stress in cells and also provide ultra violet protection towards ionising radiation.[7] Stable nitroxide species, which have been implemented in making biophysical probes, also identified as novel antioxidants. Nitroxides are readily reduced to the hydroxylamine through one-electron-transfer reactions.[14] Stable nitroxide species were reported as SOD mimics and help in the dismutation of active O_2 species through two different catalytic pathways such as reductive and oxidative mechanisms.[15] Recently, a novel antioxidant species has been developed in the treatment of ischaemic reperfusion injury when attaching an isoindoline nitroxide moiety with structure of edaravone.[16]



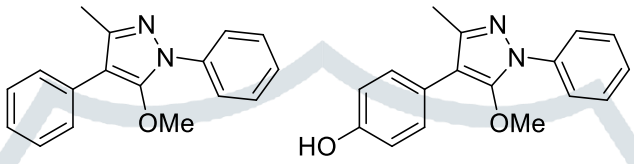
6

Figure 6: 5-(3-methyl-pyrazol-5-ol-1-yl)-1, 1, 3, 3-tetramethylisoindolin-nitroxide

James R Walker et al studied very beneficial pathophysiological target in an acute phases of ischaemia in which cells are protected from ROS. Nitroxide antioxidant moiety 5-carboxy-2-yloxyl-tetramethylisoindolin derivative (CTMIO) combines with edaravone framework that free radical scavenger used in ischaemia patients in Japan, which exhibited a tremendous decrease in cell death in ischaemic rat atrial cardiomyocytes cells after prolonged ischaemia. In treatment with nitroxide with respect to cell death the increased rate of protective effect was observed**.** Edaravone derivative **6** exhibited the lowering in cell death after 12.0 hrs

modular ischaemia simulation, which was significant higher than that of edaravone itself. The methoxy-amine derivative and edaravone combined showed negligible variation between the extents of reduction in cell death. Therefore, this potent antioxidant herein exhibited promise in the cardiovascular ischaemic diseases treatment. Further study of the derivative **6** as an effective anti ischaemic stroke agents is in progress.

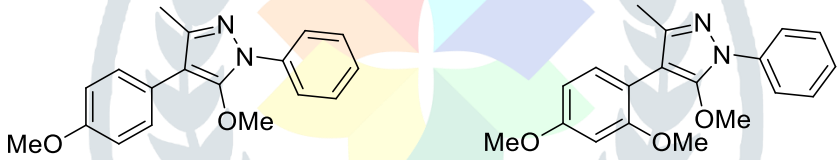
It was also suggested that modification of the carbonyl group of Edaravone to methyl oxy group under pathophysiological condition in the presence of enzyme demethylases. In this methyl oxy is changed to hydroxyl group which will produce better candidates for analysis with the receptor. Resulting modification was shown in the following compounds **(7)** and **(8)** in figure 7.[20]



7 **8**

Figure 7: Derivatives of Edaravone with methoxy group

The number of methyl oxy groups increased that also increases anti-cancer activity, as shown in compound **(9)** and **(10)** in figure 8.



9 **10**

Figure 8: Derivatives of Edaravone with enhanced anticancer activity

Derivatives **(11)** and **(12)** showed potent anticancer activity. The compound in structure (**11)** due at ortho position ethereal group showed good bioactivity towards inhibiting A549 cells due to the increase in lipophilicity also reduce steric hindrance. The compounds in structure (**12)** and (**13)** demonstrated highest anticancer activity. The compound in figure (**14)** showed the highest activity among the carboxylic acid amides with a good electron withdrawing substituent shows enhanced lipophilicity, good enduring power for metabolic destruction as a halogen bond present and also the carboxyl-amide linkage is considered valuable for the action against lung cancer. The compounds (**14)** and (**15)** showed the most free radical scavenging activity because of multiple positions where scavenging can take place and the transfer of proton from OH and NH groups. Thus, the compounds in Figure (**13)**, (**14)**, (**15)** exhibited superior anticancer activity and Derivatives **(11)** and **(12)** and (**15) e**xhibited best antioxidant activity.

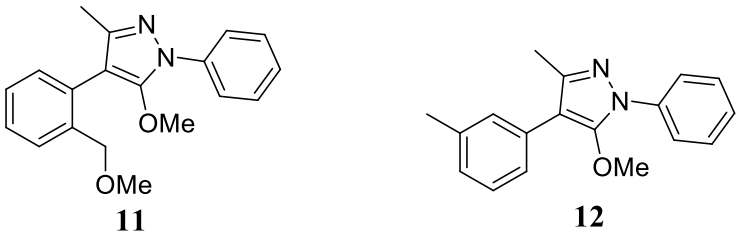


Fig. 9: Potent anticancer Edaravone derivatives.

The compounds **(13)**, **(14)** and **(15)** showed potent antioxidant activity.

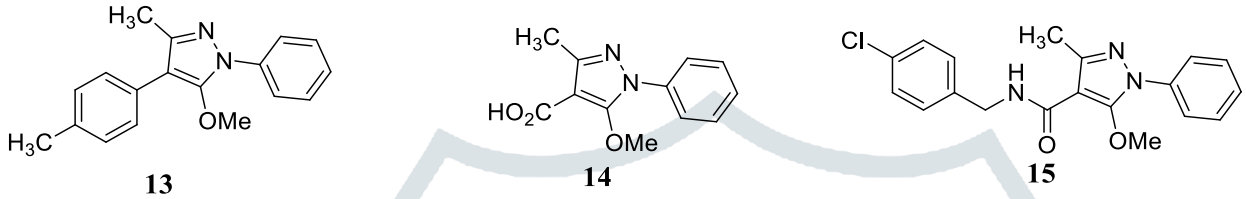


Figure 10: Potent Antioxidant Edaravone derivatives

This compound will show an antioxidant activity. Analysis of these compounds will be carried out to check its antioxidant activity by using methods such as DPPH compound (2, 2-di-phenyl-1-picrylhydrazyl) method, ABTS (2, 2'-azinobis-ethylbenzthiazoline)-6-sulfonic acid)) method, FRAP (ferric ion reducing the antioxidants power) method, HORAC (hydroxyl radical averting capacity) assay, TRAP (total peroxyl radical trapping antioxidant parameter) assay, lipid peroxidation inhibition assay, CUPRAC (cupric reducing antioxidant power) assay.

Conclusion

Number of patients having cardiovascular and neurodegenerative disease are increased, and those will benefit from antioxidant treatment in the coming future. The inhibition of reactive oxygen species induced stress and the recovery from reperfusion injury is extremely important. Edaravone is the only neurovascular protection agent available at present to achieve this effect. Furthermore, edaravone derivatives enhance the efficacy of antioxidant. However, the currently available treatments and the related information to exhibit potential of edaravone are inadequate.

REFERENCES

1. Sonia Anand, Salim Yusuf, Srinath Reddy SÔ. Global Burden of Cardiovascular Diseases Part I: General Considerations, the Epidemiologic Transition, Risk Factors, and Impact of Urbanization. *Circulation*. 2016;(104):2746-2753.

2. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies.

Circulation. 2001;104(23):2855-2864.

3. Badimon L, Padró T, Vilahur G. Atherosclerosis , platelets and thrombosis in acute ischaemic heart disease. *Eur Hear J Acute Cardiovasc Care*. 2012;1(1):60-74.

4. Florence TM. The role of free radicals in disease. *Aust N Z J Ophthalmol*. 1995;23(1):3-7.

5. Niki E. Do free radicals play causal role in atherosclerosis? Low density lipoprotein oxidation and vitamin E revisited. *J Clin Biochem Nutr*. 2011;48(1):3-7.

6. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 2010;4(8):118-126.

7. Krishna M.C, DeGraff W, Hankovszky O.H. Studies of structure-activity relationship of nitroxide free radicals and their precursors as modifiers against oxidative damage. *J Med Chem*. 1998;41(18):3477-3492.

8. Kollmar R, Schwab S. Ischaemic stroke : acute management , intensive care , and future perspectives. *Br J Anaesth*. 2007;99(1):95-101.

9. Lapchak PA. A critical assessment of edaravone acute ischemic stroke efficacy trials : is edaravone an effective neuroprotective therapy ? 2010:1753-1763.

10. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. *Eur J Cancer*. 1996;32A(1):30-38. doi:10.1016/0959-8049(95)00531-5.

11. Aruoma O.I. Free Radicals , Oxidative Stress , and Antioxidants in Human Health and Disease. *J Am Oil Chem Soc*. 1998;75(2):199-212.

12. Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging*. 2007;2(2):219-236.

13. Sindhi V, Gupta V, Sharma K, Bhatnagar S. ScienceDirect Potential applications of antioxidants: A review. *J Pharm Res*. 2013;7:3-10.

14. Krishna MC, Russo A, Mitchell JB, Goldstein S, Dafni H, Samuni A. Do nitroxide antioxidants act as scavengers of $O2^{-}\cdot$ or as SOD mimics? *J Biol Chem*. 1996;271(42):26026-26031.

15. Kikuchi K, Tancharoen S, Takeshige N. The efficacy of edaravone (radicut), a free radical scavenger, for cardiovascular disease. *Int J Mol Sci*. 2013;14(7):13909-13930.

16. Walker JR, Fairfull-smith KE, Anzai K. Edaravone containing isoindoline nitroxides for the potential treatment of cardiovascular ischaemia. *Med Chem Commun*. 2011;2:436-441.

17. Higashi Y, Jitsuiki D, Chayama K, Yoshizumi M. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a novel free radical scavenger, for treatment of cardiovascular diseases. *Recent Pat Cardiovasc Drug Discov*. 2006;1(1):85-93.

18. Nakagawa H, Ohyama R, Kimata A, Suzuki T. Hydroxyl radical scavenging by edaravone derivatives : Efficient scavenging by 3-methyl-1- (pyridin-2-yl) -5-pyrazolone with an intramolecular base. *Bioorg Med Chem Lett*. 2006;16:5939-5942.

19. Chegaev K, Cena C, Giorgis M, et al. Edaravone Derivatives Containing NO-Donor Functions. *J Med Chem*. 2009;52:574-578.

20. Polkam N, Ramaswamy VR, Rayam P. Synthesis, molecular properties prediction and anticancer, antioxidant evaluation of new edaravone derivatives. *Bioorg Med Chem Lett*. 2016.;1-8.