Free Radical Biology and Medicine 62 (2013) 145–156

Contents lists available at SciVerse ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Nitrone-based therapeutics for neurodegenerative diseases: Their use alone or in combination with lanthionines

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ARTICLE INFO

Available online 16 February 2013

Keywords:
Nitrones
Glioma
Glioblastoma multiforme
Cancer
Dementia
Septic shock
Malaria dementia
Aging
Lanthionine
PBN
NXY-059
OKN-007
Spin trapping
Free radicals

ABSTRACT

The possibility of free radical reactions occurring in biological processes led to the development and employment of novel methods and techniques focused on determining their existence and importance in normal and pathological conditions. For this reason the use of nitrones for spin trapping free radicals became widespread in the 1970s and 1980s, when surprisingly the first evidence of their potent biological properties was noted. Since then widespread exploration and demonstration of the potent biological properties of phenyl-*tert*-butylnitrone (PBN) and its derivatives took place in preclinical models of septic shock and then in experimental stroke. The most extensive commercial effort made to capitalize on the potent properties of the PBN-nitrones was for acute ischemic stroke. This occurred during 1993–2006, when the 2,4-disulfonylphenyl PBN derivative, called NXY-059 in the stroke studies, was shown to be safe in humans and was taken all the way through clinical phase 3 trials and then was deemed to be ineffective. As summarized in this review, because of its excellent human safety profile, 2,4-disulfonylphenyl PBN, now called OKN-007 in the cancer studies, was tested as an anti-cancer agent in several preclinical glioma models and shown to be very effective. Based on these studies this compound is now scheduled to enter into early clinical trials for astrocytoma/glioblastoma multiforme this year. The potential use of OKN-007 in combination with neurotropic compounds such as the lanthionine ketamine esters is discussed for glioblastoma multiforme as well as for various other indications leading to dementia, such as aging, septic shock, and malaria infections. There is much more research and development activity ongoing for various indications with the nitrones, alone or in combination with other active compounds, as briefly noted in this review.

Contents

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http://dx.doi.org/10.1016/j.freeradbiomed.2013.01.033

Why should nitrones be considered potential therapeutics?

It is important to ask the question, why are the PBN (α-phenyl-*N*-*tert*-butylnitrone) nitrones considered potential therapeutics? The most important reason is that PBN and some of its congeners have been found to have extremely potent biological activity in several experimental biological systems. Historically, in the late 1960s and early 1970s the nitrones became important agents to aid in the identification of highly unstable free radical intermediates in chemical reactions because they could react with and stabilize free radicals by what was commonly known as a spin trapping reaction. To the surprise of some chemists the nitrones PBN and DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) also proved to be useful to trap free radicals in biochemical systems. Their use was then extended to biological systems [1]. It was found that extending their use to biological systems became problematic for their intended purpose of identifying the free radical intermediates because the spin adduct nitroxide was chemically reduced by the reductive biological processes and rendered paramagnetic silent. However, it was discovered in several instances that the nitrones had potent biological activity in many biological systems [1,2]. This report presents a summary of the past, present, and future prospects of research and development focused on the therapeutic potential of nitrones.

Spin trapping—history and general considerations

The spin trapping method, named by E.G. Janzen, was developed independently by various researchers in 1968 and 1969 [3–8] to extend the limits of electron spin resonance (ESR) spectroscopy so that lower concentrations of free radicals could be detected indirectly. This method involves the trapping of reactive short-lived free radicals by a diamagnetic spin trap compound via an addition reaction to produce a more stable free radical product or spin adduct. The spin adduct that is formed is paramagnetic and has an ESR spectrum with a hyperfine splitting constant and *g* value characteristic of the type of reactive free radical trapped. Thus, the structure of the radical trapped can usually be deduced, although the most difficult aspect of the spin trapping technique is the correct assignment of the nitroxide spectrum to the original radical species.

Nitrones are commonly used spin trapping agents in biological systems. The addition of a reactive free radical ($R^{\bullet}$) with a nitrone spin trap results in the formation of a nitroxide (see Fig. 1). Nitroxides are stable free radicals because of the resonance stabilization of the unpaired electron between the nitrogen and the oxygen of the nitroxyl functional group [9]. The most stable nitroxides are those with inert functional groups attached to the nitrogen atom, such as methylated carbon atoms [10–12]. Examples of commonly used nitrones are PBN [3] and the cyclic nitrone DMPO [8,13–15]. Interesting derivatives of PBN bound to cyclodextrins, such as permethylated cyclodextrin or 2,6-di-*O*-Me-β-cyclodextrin-grafted PBN, have been found to trap carbon- and oxygen-centered free radicals with enhanced ESR signal intensities [16]. Recently, DMPO and other cyclic nitrones (e.g., AMPO, EMPO, 5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide (DEPMPO)) have been assessed for their abilities to trap reactive nitrogen species, such as $^{\bullet}NO_2$, $ONOO^-$, and $ONOOCO_2^-$ [17,18]. Improvements in nitrones for the trapping of superoxide ($O_2^{\bullet -}$) include derivatives of the DEPMPO nitrone, 5-diethoxyphosphoryl-4-hydroxymethyl-5-methyl-1-pyrroline-*N*-oxide, the β-cyclodextrin–cyclic nitrone conjugate 5-*N*-β-cyclodextrin–carboxamide-5-methyl-1-pyrroline *N*-oxide [19], a cyclic nitrone conjugate of calyx[4]pyrrole (CalixMPO) [20], and a 4-furoxanyl nitrone (FxBN) [21]. For the detection of mitochondrial-generated free radicals, a novel cyclic nitrone spin trap containing a phosphonium cation, [4-(2-methyl-1-oxy-3,4-dihydro-2*H*-pyrrole-2-carbonyloxy)-butyl]-triphenylphosphonium bromide, for trapping superoxide and hydroxyl radicals [22], and a nitrone-containing *N*-arylpyridinium salt for trapping carbon-centered radicals [23] could be useful.

The type of spin trap used is an important factor in determining how informative and sensitive the spin trapping technique may be for a given free radical species. It is important to emphasize that no single spin trap is optimal for the trapping of all types of reactive free radicals. The main advantages of using nitrone spin traps are: (i) they are less sensitive to light, oxygen, or water vapor; (ii) they are soluble in a large number of solvents at fairly high concentrations (~0.1 M); and (iii) spin adducts are considerably more stable because a carbon atom separates the nitroxide functional group from the trapped radical species [8,24]. The use of nitrone compounds also has some disadvantages such as: (i) the information regarding the nature and structure of the trapped species is difficult to obtain from the spectrum of the spin adduct, e.g., ESR spectra PBN spin adducts have characteristically small $a_H\beta$ (β-hyperfine splitting constant) values with little variation in magnitude among free radical species, although the magnitude of the β-hyperfine splitting is enhanced with the cyclic nitrone DMPO, and (ii) photolysis of PBN in certain solvents (e.g., tetrahydrofuran) rapidly produces spin adducts derived from solvent radicals [8,16]. Reviews of interest

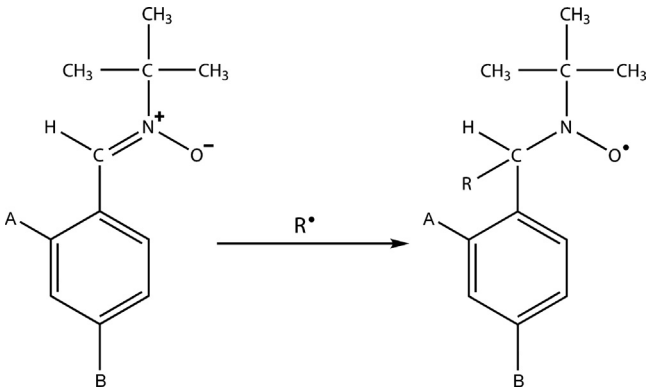

Fig. 1. Spin trapping reaction in which a free radical has reacted with a general PBN-type nitrone to form a spin adduct. In the case of PBN, A=B=H, and with OKN-007, A=B=SO_3Na_2.