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Selenium-Based Antihypertensives
Rationale and Potential

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Abstract

Selenium, long recognised as an important 'dietary antioxidant', is now known to be an essential component of the active sites of a number of enzymes, including the glutathione peroxidase selenoenzyme family which scavenge hydroperoxides to prevent cellular damage. Dietary selenium deficiency has been linked to diseases as diverse as cancer, heart disease, arthritis and AIDS, and epidemiological evidence is now emerging for the beneficial effects of selenium supplementation. Thus, the pharmacology, biology and biochemistry of selenium metabolism have become subjects of considerable interest, which are spurring efforts to develop synthetic selenium-containing compounds as potential therapeutic agents.

Phenylaminoalkyl selenides were developed in the authors' laboratories as novel, selenium-based pharmacological agents. We demonstrated that these compounds exhibited dose-dependent antihypertensive activity in spontaneously hypertensive rats. Biochemical studies established that as a consequence of the redox properties of their selenium moieties, these phenylaminoalkyl selenides possessed the remarkable property of propagating a cycle of turnover-dependent local depletion of reduced ascorbate when processed by the key enzyme of catecholamine metabolism, dopamine-β-monooxygenase.

On the basis of inductively coupled plasma/mass spectroscopic analyses, corroborated by operant behaviour and locomotor activity investigations, an orally-active phenylaminoalkyl selenide with restricted CNS permeability was successfully developed. To our knowledge, this compound – 4-hydroxy-α-methylphenyl-2-aminoethyl selenide – is the first orally active, selenium-based antihypertensive compound ever reported. In the future, we anticipate more widespread efforts to incorporate selenium into rationally designed pharmaceutical agents, with the goal of developing novel compounds which may be of therapeutic benefit toward a variety of human diseases.

1. The Essential Biological Role of Selenium

The pharmacology, biology and biochemistry of selenium are subjects of intense current interest, particularly from the viewpoint of public health. Selenium has long been considered to be an important 'dietary antioxidant', and selenium deficiency has been linked to a number of disorders such as heart disease, cancer, diseases of the liver and pancreas, osteoarthritis and, recently, AIDS.[1-8] In December, 1996, a report by Clark et al.[9] on the beneficial effects of selenium supplementation attracted a substantial amount of media interest. These investigators carried out a long term, double-blind, placebo-controlled study on 1312 patients, and found that supplementation with 200μg of selenium reduced the incidence of lung, colorectal and

prostate cancer and the total cancer mortality. Indeed, the United States National Research Council has had a long-standing recommended dietary allowance (RDA) of 55 to 70 μg for selenium.[10]

At the molecular level, much attention has centred on selenium's essential presence in the selenocysteine residue of the glutathione peroxidase selenoenzyme family.[11,12] These enzymes, in the presence of glutathione, scavenge hydroperoxides to prevent cellular damage. Selenium also plays an essential role in the activity of the enzyme iodothyronine 5′-deiodinase, and several other mammalian selenoproteins (e.g. 'selenoprotein P' and 'selenoprotein W') have been recently identified and characterised.[11] Selenocysteine is now commonly referred to as the 'twenty first amino acid', and has begun to be identified as such in popular biochemistry textbooks.[13]

A remarkable genomic effect of selenium deficiency at the molecular level was recently reported by Beck et al.[14] These investigators demonstrated that a nonvirulent strain of Coxsackievirus mutates in selenium-deficient mice to produce virulent virus, capable of infecting other mice which had been fed normal diets; strikingly, in every case a single genetic variant of the virus was produced, altered in precisely the same 6 nucleotide positions.

What is the underlying mechanism by which a selenium-deficient diet can lead to selection of a single, stable cardiovirulent Coxsackievirus strain? This and related questions have been discussed by the editors of *Nature Medicine* in an overview which accompanied the paper by Beck at al.[14] Clearly, such issues cannot be fully addressed until much more is known at the molecular level regarding the biochemistry and pharmacology of selenium and its metabolites.

2. Pharmacologically Active Seleno-Organic Compounds

The impressive and rapidly emerging body of epidemiological, biochemical and pharmacological evidence for the beneficial effects of selenium has spurred increasing efforts to delineate the biochemical pathways of selenium assimilation and metabolism. A clear measure of this interest is the fact that more than 30 active projects on selenium metabolism are currently listed in the Grants Database of the United States National Institutes of Health. What is less widely appreciated is that there have also been a number of ongoing efforts over the past 2 decades aimed at developing synthetic selenium-containing compounds as potential therapeutic agents. These efforts have been met with varying degrees of success and periodic review articles have summarised the synthesis and properties of many seleno-organic compounds prepared as potential pharmaceuticals.[15-17] In general, as Parnham[18] has observed, while a great many such seleno-organic compounds have been synthesised, only rarely have new entities with genuine therapeutic potential emerged from these efforts.

One example of a well described seleno-organic compound which has undergone a substantial amount of pharmacological testing is ebselen (2-phenyl-1,2-benzoisoselinazol-3(2H)-one). This compound was designed to mimic the enzymatic activity of glutathione peroxidase, and it has been reported that ebselen is undergoing phase III clinical testing for inhibition of subarachnoid haemorrhage and stroke.[18] It has also been reported that ebselen increases the levels of reduced glutathione, glutathione reductase and heat shock proteins in cultured cardiac myocytes, resulting in increased resistance to oxidative injury.[19] Two additional examples are selenazofurin, as an antineoplastic and antiviral agent, and selenotifen, as an antiallergic agent. Although these compounds have not been as extensively investigated, both are pharmacologically active organoselenium compounds which are significantly more active than their corresponding sulphur analogues.[15]

3. Phenylaminoalkyl Selenides as Antihypertensives

Our own work with selenium-based pharmacological agents began with synthesis of the novel compound phenylaminoethyl selenide (PAESe) as a potential alternate substrate for the key enzyme of catecholamine metabolism, dopamine-β-mono-

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oxygenase (DBM). DBM is an attractive target point for modulation of peripheral adrenergic activity, and we and others had demonstrated that a number of DBM-directed inhibitors and pseudosubstrates have been shown to exhibit antihypertensive activity.[20,21] We reasoned that DBM should be capable of oxygenating phenylaminoalkyl selenides with high facility, and we anticipated that the expected selenoxide products could well exhibit unique reactivity and redox properties. Indeed, we confirmed that PAESe is an excellent substrate for DBM and that enzymatic oxygenation produces phenyl-2-aminoethyl selenoxide (PAESeO) via the normal ascorbate-dependent reductive oxygenation pathway of DBM catalysis.[22]

What is remarkable about the processing of phenylaminoalkyl selenides by DBM is the unique ability of these compounds to initiate and sustain a cycle of local depletion of reduced ascorbate within adrenergic vesicles.[23-25] Initially, DBM present within the vesicle converts the phenylaminoalkyl selenides to the corresponding selenoxide products in a facile process proceeding via the normal, ascorbate-dependent oxygenation pathway of DBM catalysis. The product selenoxides are then nonenzymatically reduced back to the corresponding selenides, with the concomitant and stoichiometric oxidation of reduced ascorbate present in the vesicle to fully oxidised ascorbate. This selenide/ascorbate cycle is a localised process since DBM is present only in these vesicles and reduced ascorbate does not cross the vesicle membrane.

While adrenergic vesicles possess a cytochrome b_{561}-dependent ascorbate recycling system, this system can only recycle semidehydroascorbate, which is generated during DBM turnover, and cannot recycle the fully oxidised ascorbate produced by the non-enzymatic selenoxide/ascorbate reaction. Thus, the net result of selenide processing in the vesicle is the effective local depletion of reduced ascorbate – an essential cofactor for DBM – and the inhibition of norepinephrine production (fig. 1). We have demonstrated this turnover-dependent ascorbate depletion process both *in vitro* and in chromaffin granule ghosts,[22-25] and we have established cellular and vesicular uptake of the selenides.[26] Moreover, as predicted on the basis of the redox potentials of selenoxides versus sulfoxides, we have demonstrated[23,24] that while sulfur-containing analogues undergo DBM-catalysed oxygenation they are not capable of propagating such a cycle of ascorbate depletion. Thus, it is clear that the ability of these selenides to effect turnover-dependent depletion of local reduced ascorbate is a direct consequence of the redox chemistry of the selenium moiety present in these compounds.

In vivo pharmacological testing of PAESe confirmed that this compound exhibits dose-dependent antihypertensive activity when administered intraperitoneally to spontaneously hypertensive rats, and we have provided evidence that the adrenergic nerve terminal is indeed the pharmacological site of action of PAESe *in vivo*.[27] However, as is true for other peripherally acting pharmacological agents,[28-30] the CNS permeability of PAESe is of significant concern, since undesirable adverse effects can often result from CNS penetration. Moreover, it is highly desirable from a therapeutic perspective that pharmacological agents used to treat chronic diseases such as hypertension be orally active. We therefore proceeded to couple 3 distinct but complimentary approaches – biochemical reasoning, state-of-the-art analytical chemistry, and operant behavioural analyses – for the purpose of developing a selenium-based antihypertensive agent which would exhibit both oral activity and restricted CNS permeability.

Briefly, our approach was as follows:[31]

- First, we employed inductively coupled plasma/mass spectroscopic (ICP/MS) analysis of plasma samples to determine the pharmacokinetic parameters for selenide compounds following intravenous administration to anaesthetised rats. Analysis of the data using a 2-compartment pharmacokinetic model established very rapid initial clearance and a short beta-elimination half-life from blood.
- Next, an oxidative procedure for digestion and processing of tissue samples was then devel-

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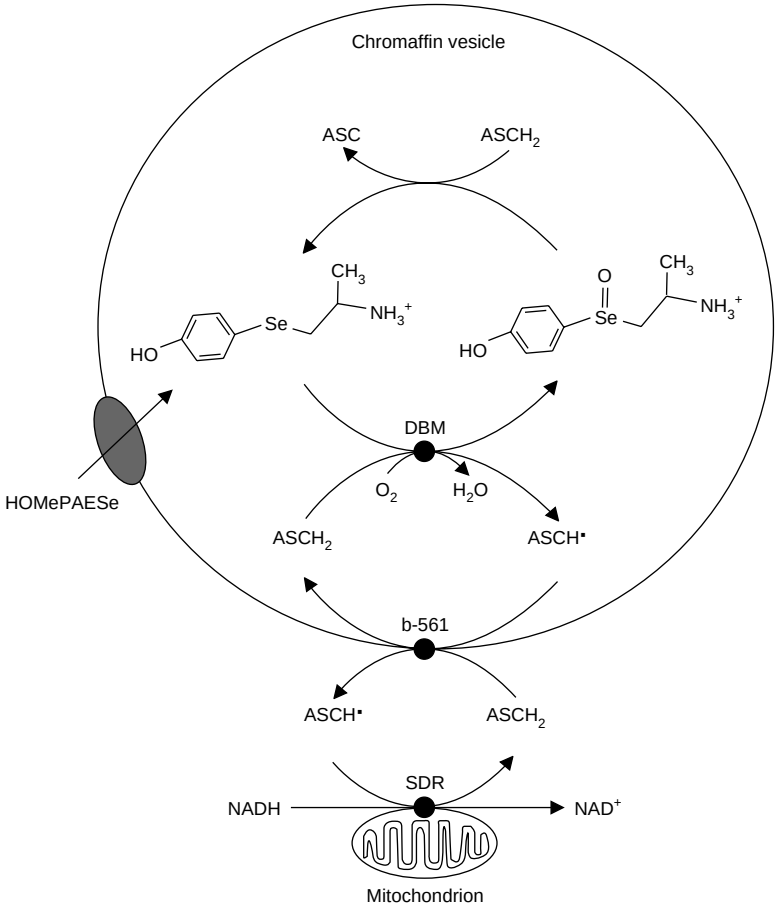

Fig. 1. Ascorbate oxidation cycle within the chromaffin vesicle. As a consequence of the redox properties of the product selenoxide, the DBM-catalysed oxygenation of HOMePAESe initiates and sustains a cyclic path of selenoxide reduction at the expense of reduced ascorbate which continues until local supplies of reduced ascorbate are depleted.[22-24] **ASC** = oxidised ascorbate; **$ASCH_2$** = reduced ascorbate; **ASCH•** = semidehydroascorbate; **b-561** = cytochrome b_{561}; **DBM** = dopamine-β-monooxygenase; **HOMePAESe** = 4-hydroxy-α-methyl-phenyl-2-aminoethylselenide; **NAD^+** = (oxidised) nicotinamide adenine dinucleotide; **NADH** = (reduced) nicotinamide adenine dinucleotide; **SDR** = semidehydroascorbate.

oped in order to obtain ICP/MS data on the tissue distributions of selenium-containing metabolites following administration of selenide compounds. The results clearly demonstrated that aromatic ring hydroxylation of the selenides results in a marked reduction in brain levels of selenium-containing metabolites.

- The comparative effects of PAESe derivatives on locomotor activity and operant behaviour were then investigated. The results fully corroborated our analytical data, thus confirming the pharmacological relevance of the ICP/MS results and providing a compelling basis for drug design.

On the basis of this multidisciplinary approach, we successfully demonstrated that the novel compound 4-hydroxy-α-methyl-phenyl-2-aminoethyl selenide exhibits both restricted CNS permeability and oral antihypertensive activity in spontaneously hypertensive rats. To our knowledge, this compound is the first orally active selenium-based antihypertensive compound ever reported.

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4. Future Potential of Selenium-Based Therapeutic Agents

In our view, there is considerable merit in the concept of harnessing the unique chemical and biochemical properties of selenium for the rational development of novel therapeutic agents. As mentioned above, it has now become quite apparent that selenium plays an essential role in several key metabolic processes, and that selenium deficiency can have severe consequences to human health. Clearly, the metabolic pathways for selenium assimilation and elimination must be active in normal, healthy individuals. Thus, the selenium moiety in selenium-based therapeutic agents does not represent, *per se*, a metabolically aberrant chemical entity, and is probably more 'biocompatible' from a metabolic viewpoint than some of the complex heterocyclic functionalities present in a number of modern pharmaceutical agents.

The notion of developing a selenium-based pharmaceutical agent might well raise initial concerns about possible toxicity at the dose levels needed for therapeutic action. There is certainly extensive literature describing the characteristics of chronic selenium poisoning.[32] However, as recently re-emphasised by the World Health Organization, it must be recognised that observed deleterious effects of selenium reflect the particular chemical form of the selenium compound to which exposure has been excessive.[32]

In this regard, we and others have reported no evidence of selenium toxicity at the dose levels of pharmacological activity for appropriately designed selenium-based compounds.[18,27] Moreover, it is striking that recent studies have shown that selenium reduces the nephrotoxicity and bone marrow suppression induced by cisplatin in cancer patients.[33] Similarly, it is known that selenium can prevent the toxic effects of several heavy metals and arsenic.[34,35] We are therefore optimistic that our successful development of an orally active selenium-based antihypertensive agent will encourage more widespread efforts to incorporate selenium into rationally designed pharmaceutical agents, with the goal of developing novel compounds which may be of therapeutic benefit toward a variety of human diseases.

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