Chemistry Proceedings

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Paradoxical Behavior of Organodiselenides: Pro-Oxidant to Antioxidant †

Vishwa V Gandhi [1,2], K. I. Priyadarsini [3] and A. Kunwar [1,2,*]

[1] Homi Bhabha National Institute, Anushaktinagar, Mumbai 400 094, India; vvgandhi@barc.gov.in
[2] Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Mumbai-400085, India
[3] UM-DAE Centre for Excellence in Basic Sciences, University of Mumbai, Kalina Campus, Santacruz (E), Mumbai 400098, India; k.indira@cbs.ac.in
* Correspondence: kamit@barc.gov.in; Tel.: +91-022-2559-2352

† Presented at the 1st International Electronic Conference on Catalysis Sciences, 10–30 November 2020; Available online: https://eccs2020.sciforum.net.

Published: 10 November 2020

Abstract: Over the years, organodiselenides have emerged as the biologically relevant class of molecules. On one hand, such compounds are known for pro-oxidant effects leading to toxicity in biological systems. On the other hand, there are growing evidences about their bio-mimetic activities as catalysts such as glutathione peroxidase (GPx)-like activity. Our recent work has explored this paradoxical behavior of diselenides in developing antioxidants and/or anticancer agents. For this, a number of alkyl and aryldiselenides have been evaluated in different biological models. The results have shown that aryl diselenides specifically pyridinediselenides alter the ratio of the intracellular thiol redox pairs (GSH and GSSG) towards reduction (antioxidant) rather than oxidation(pro-oxidant) to protect normal cells against radiation damage as well asto induce cytotoxicity in tumor cells. Further, these studies have also postulated that intracellular redox state, level of thioredoxin reductase (TrxR) and reductive intermediates (like selenol and/or selone) might play a very important role in the manifestation of the toxicities of aryl diselenides in cells

Keywords: organodiselenides; glutathione peroxidase; thioredoxin reductase; reductive stress; cytotoxicity

1. Introduction

Glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) are the two most important seleno-enzymes present in mammalian cells for maintaining redox homeostasis [1]. GPx catalyses the reduction of toxic hydro/lipid peroxides into non-toxic water molecules by using thiol (GSH) as a cofactor. Similarly, TrxR catalyses the reduction of oxidized thioredoxin (Trx) into reduced form by using NADPH as a cofactor. The catalytic activities of GPx and TrxR are governed by selenocysteine, a selenium containing aminoacid present in their active sites [1]. The natural existence of such selenoenzymes has been the driving force for the design, synthesis and evaluation of organoselenium compounds as pharmacological agents [2]. In last one or decades, several reports have appeared in literature on the synthesis as well as biological activities of both alkyl and aryl diselenides [3,4]. These studies have together confirmed the ability of diselenides to mimic GPx-like activity and to act as the substrates for TrxR under cell free conditions. However, under cellular conditions, diselenides can manifest antioxidant or pro-oxidant activity depending on their chemical forms, concentration and redox state of the host cells [3,4]. Pyridine is an important chemical form present in several of biological molecules like vitamin, nucleic acid, etc. Additionally, it has been shown that diselenides containing heterocyclic ring (e.g., pyridine) are better catalysts than the compounds containing simple aryl groups e.g., diphenyl diselenide (Ph_2Se_2) [5,6]. On similar lines, we have recently reported

the synthesis, purification, structure and biological activities of dipyridine diselenide (Py_2Se_2) and its amide derivative dinioctinamide diselenide (Nic_2Se_2) [5–9]. The present report discusses the salient findings of the biological activities of these two molecules so as to improve our understanding towards the toxicology of organodiselenides. The chemical structures of Py_2Se_2 and Nic_2Se_2 are given in Scheme 1.

(Py_2Se_2)
Dipyridine dislenide

(Nic_2Se_2)
Dinioctinamide diselenide

Scheme 1. Chemical structures of Py_2Se_2 and Nic_2Se_2.

2. Bio-Chemical Activities under Cell Free Conditions

2.1. GPx-Like Activity

The GPx like activity of organoselenium compounds has been evaluated by coupled NADPH assay. The principle of this assay is the reduction of peroxide (e.g. cumene hydroperoxide or H_2O_2) by organoselenium compounds using GSH and NADPH as cofactors. Accordingly, the quantitative determination of the decay of NADPH or GSH by sensitive analytical techniques like spectrophotometer or HPLC or NMR gives the measure of GPx-like activity of the test compound [5–9]. By using these techniques, both Py_2Se_2 and Nic_2Se_2 have been evaluated for GPx like activity under cell free conditions and compared with a standard diselenide like Ph_2Se_2 [5–9]. The results have indicated that their activities followed the order Nic_2Se_2>Py_2Se_2>Ph2Se2 (Figure 1). The higher GPx like activity of Nic_2Se_2 was attributed to the presence of non-bonding interaction between Se with N in this molecule [9]. As expected, GPx-like activity of a diselenide can be initiated either through its reduction into selenol (RSeH) by GSH or its oxidation into selenenic acid (RSeOH) by peroxide [9]. Enzyme kinetic analysis of Nic_2Se_2 has revealed that GPx cycle of Nic_2Se_2 is initiated predominately by its reduction with GSH. Further, it is also shown that unlike simple aryl diselenide (Ph_2Se_2), the reduction of Nic_2Se_2 having pyridine ring first forms selenol (RSeH) which is immediately converted into stable selone (RC=Se) and this intermediate can further take part in scavenging of reactive oxygen species (ROS) directly or indirectly through entry into GPx cycle [9].

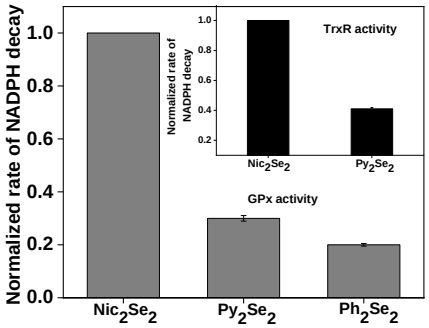


Figure 1. Plot shows GPx activity of different organodiselenides in terms of the rate (ΔOD/min) of decay of NADPH under cell free conditions. Inset of the figure shows the TrxR activity in terms of the rate (ΔOD/min) of decay of NADPH using different organodiselenides as substrates under cell free conditions. In both figures, the rate NADPH decay is normalized with respect to that of Nic_2Se_2, the most active derivative. The catalyst (organoselenium compound) used for GPx and TrxR assay was 10 μM and 25μM respectively.

2.2 Substrate of TrxR

Several studies have shown that TrxR can reduce the organodiselenides in to corresponding selenols and/or selone [3,4,10–12]. Accordingly, Nic_2Se_2 and Py_2Se_2 have been evaluated for their ability to undergo TrxR mediated reduction. This possibility can be checked by simply following the decay of NADPH in presence of test compound and the enzyme TrxR. Alternatively; the test compound can also be evaluated for its competitive inhibitory effect on TrxR mediated reduction of DTNB (dithionitrobenzoic acid) into TNB (thionitrobenzoic acid). Interestingly, at an equimolar concentration, both Nic_2Se_2 and Py_2Se_2 have been found to increase the decay of NADPH or to inhibit TNB formation in TrxR activity assay reactions suggesting that these molecules can undergo TrxR mediated reduction [10,11]. Notably Nic_2Se_2 appeared to be more potent substrate than Py_2Se_2 for the mammalian TrxR (Figure **1).**

3. Comparative Cytotoxicity and Redox Modulation in Cells

Having understood the catalytic activities of Py_2Se_2 andNic_2Se_2, these molecules have been investigated for cytotoxicity in cellular models [7,8,10,11]. The Table 1 shows the IC_{50} values of Py_2Se_2 and Nic_2Se_2 to induce cytotoxicity in various cell types by MTT assay. It can be seen that Py_2Se_2 is more toxic than Nic_2Se_2 in all the cell types investigated.

Table 1. $IC_{50}$of organodiselenides in cell lines [8,10,11].

Compounds	CHO (Normal ovary epithelium)	WI38 (Normal lung fibroblast)	A549 (Lung carcinoma)	MCF7 (Breast carcinoma)
2,2·-dipyridyl diselenide (Py_2Se_2)	~6 μM	~8 μM	~5 μM	~5 μM
2,2′-diselenobis-[3-amidopyridine] (Nic_2Se_2)	>100 μM			~70 μM

In general, the cytotoxic effects of alkyl and aryl diselenides have been correlated with their pro-oxidant effects or abilities to modulate the intracellular redox environment (GSH/GSSH) towards oxidation [3,4]. Accordingly, Py_2Se_2 and Nic_2Se_2 were expected to show similar effects in cellular systems. However, results have shown that treatment of CHO and A549 cells with Nic_2Se_2 and Py_2Se_2 respectively in the nearly identical concentration range (10–25 μM) resulted in a significant decrease in basal ROS level and a concurrent increase in total thiol content as well as increase in the ratio of glutathione couple (GSH/GSSH) (Figure 2) [10,11]. Additionally, treatment with Py_2Se_2 or Nic_2Se_2 led to significant increase in the transcript level of γ-GCL, a rate limiting enzyme involved in GSH biosynthesis [10,11]. Thus together these results have suggested that Py_2Se_2 or Nic_2Se_2 induces reductive environment in cells. However, the extent of reductive state in terms of the fold change in the ratio of GSH/GSSG was significantly higher in Py_2Se_2 treated cells as compared toNic_2Se_2 treated cells [10,11]. Further reductive stress in Py_2Se_2 treated A549 cells were found to be associated with DNA damage, G1 phase arrest and apoptosis [11]. On the other hand, pretreatment of CHO cells with Nic_2Se_2 showed significant protection from γ-radiation induced DNA damage and cell death [10]. Both Py_2Se_2 and Nic_2Se_2 are expected to undergo reduction within cellular environments by three pathways: 1) Futile cycle involving GSH 2) GPx cycle involving GSH and 3) as a TrxR substrate involving NADPH. Of these three pathways, the futile cycle is believed to be responsible for the pro-oxidant effects of diselenides through generation of ROS, whereas the rest two pathways can contribute to reductive environment through ROS scavenging either directly or via intermediates like selone [3,4,9,10]. Since treatment of cells with Py_2Se_2 and Nic_2Se_2 exhibited elevation rather than decrease in GSH/GSSG, the entry of Py_2Se_2 and Nic_2Se_2 into GPx and TrxR appear to the predominant pathways for their reduction within cells. At this stage, it is also important to understand the factors contributing to differential toxicity of Nic_2Se_2 versus Py_2Se_2. In this context, one of the probable factors

could be the formation of reaction intermediates. As shown in Scheme 2, the reductive pathway of Py_2Se_2 and Nic_2Se_2 forms selenol which is further converted into selone [9].

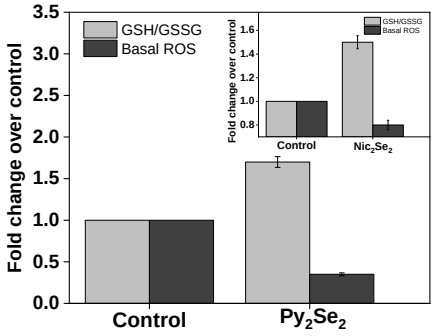
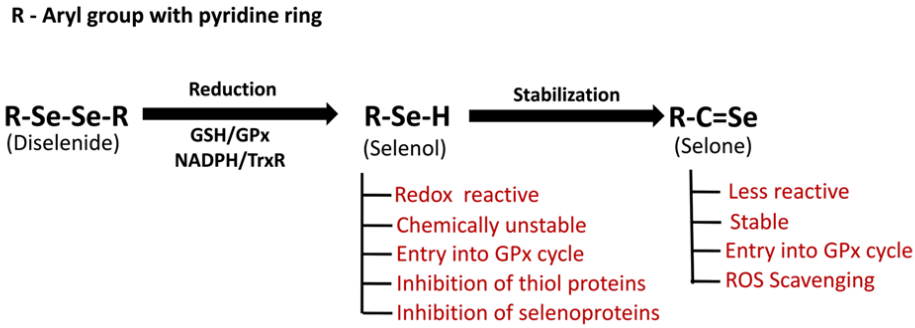


Figure 2. Plot shows fold changes in the levels of basal GSH/GSSG and ROS in A549 cells after treatment with Py_2Se_2 (10 μM) for 24 h [11]. Inset shows fold changes in the levels of basal GSH/GSSG and ROS in CHO cells after treatment with Nic_2Se_2 (10 μM) for 16 h [10]. The levels of GSH/GSSG and ROS in treatment groups are normalized with respect to control group.

Of these two intermediates, selenol is considered to be highly reactive and can react with cellular thiol or thiol containing proteins inhibiting their activities and thus can cause cytotoxicity. On the other hand, selone is more stable as well as less reactive species. Therefore, relative proportion of selenol and selone as well as stability of the latter, can dictate the toxicity of aryl diselenides containing pyridine ring. Notably our studies have shown that selone is the predominant intermediate formed during reduction of Nic_2Se_2 due to availability of selenoamide moiety providing extra stabilization to the reduced species [9]. However, in case of Py_2Se_2, there is possibility of the degradation of selone species explaining its higher toxicity in both normal and tumor cells. These assumptions are also justified considering the fact that cells treated with Py_2Se_2 showed inhibition or decrease in the activity of selenium or thiol containing proteins like GPx, TrxR, glutathione S transferase (GST), and glutathione redcutase (GR) [11]. On contrary Nic_2Se_2 treatment to cells did not show much inhibition of these proteins [10]. In addition to above discussed factors, tumor versus normal cells differ in terms of intracellular redox state as well the expression levels of TrxR (which is known to be generally over-expressed in tumor cells) and therefore these may also contribute to differential toxicity of aryl diselenides containing pyridine ring, however this needs to be validated in future studies.



Scheme 2. Scheme depicts reductive pathway of aryl (with pyridine ring) diselenide forming intermediates with their probable functions.

4. Conclusions

In conclusion, our results for the first time provided evidence that low concentrations of aryl diselenides containing pyridine ring modulates intracellular redox state towards reduction

(antioxidant) rather than oxidation (pro-oxidant) side in both normal and cancer cells. The reductive stress mediated by such compounds leads to cytotoxic or apoptotic effect in cancer cells. Additionally, cellular redox state, level of TrxR and reductive intermediates (selenol versus selone) appear to be the major determinants of the toxicity of aryl diselenides with pyridine ring. Accordingly, our future interest is to correlate the cellular speciation of diselenides with their biological activities in cells and *in vivo* model systems.

Author Contributions: Conceptualization, A.K.; methodology, V.V.G.; validation, V.V.G.; formal analysis, V.V.G. and A.K.; investigation, V.V.G. and A.K.; resources, K.I.P.; writing—original draft preparation, V.V.G. and A.K.; writing—review and editing, K.I.P. and A.K.; supervision, A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding

Acknowledgments: The authors thank Dr. V.K. Jain, Director, UM-DAE-CEBS, Dr. B.G. Singh, BARC, Dr. P.P. Phadnis, BARC and students whose names appeared as co-authors in the publications. The authors also acknowledge Dr. Awadhesh Kumar, Head, RPC Division BARC, and Dr A. K. Tyagi, Associate Director, Chemistry Group, BARC, for their support and encouragement. KIP thanks DAE for the Raja Ramanna Fellowship

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

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