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GLUTATHIONE PEROXIDASE-LIKE ACTIVITY OF DIARYL TELLURIDES.

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Abstract: 4,4'-Disubstituted diaryl tellurides showed glutathione peroxidase-like catalysis of the reduction of hydroperoxides by glutathione. The most potent compound, bis(4-aminophenyl) telluride, demonstrated 348%, 530%, 995% and 900% of the catalytic efficacy of Ebselen for the glutathione dependent reduction of H_2O_2, t-butyl hydroperoxide, cumene hydroperoxide and linoleic acid hydroperoxide, respectively.

Cells and tissues of most organisms are constantly under pressure from the oxidative environment of aerobic life. This oxidative burden arises mainly from the production of reduced oxygen metabolites (ROMs) during normal cellular metabolism.[1] Normally, the levels of these reactive oxygen metabolites are controlled by the coordinated activities of extensive intra- and extracellular antioxidant networks.[2]

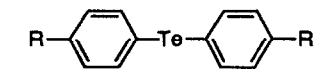
The importance of free radical-mediated tissue damage, as well as the pathological effects of the products from oxidative insult in diseases, has become increasingly appreciated during the last decades. Thus, the involvement of oxidative stress in conditions such as inflammation, atherosclerosis, shock, cataracts and reperfusion injury is generally accepted. Recent studies have also provided evidence for a controlling role of peroxidation products such as hydroperoxides and aldehydes in disease development. As a consequence, pharmaceutical companies have attempted development of antioxidant compounds as possible pharmaceutical principles for the treatment of the above diseases.

Glutathione (GSH) plays a central role in the endogenous antioxidant defence as a reducing agent and nucleophile and as a substrate for the glutathione peroxidases (GSH-pxs) and transferases.[3] Manipulation of the GSH system has proved promising and xenobiotic agents have been described which augment the biosynthesis of the tripeptide. The best studied examples of these are L-2-oxothiazolidine-4-carboxylate,[4] N-acetylcysteine (NAC)[5, 6] and glutathione esters.[7] More recently, several reports have been published on GSH-px-mimetic compounds which, like the native enzyme forms, rely on the redox cycling of selenium. The first example of such a compound was Ebselen. This substance showed antiinflammatory efficacy in a variety of model inflammations.[8, 9] We have previously reported the antioxidant capacity of diaryl chalcogenides in biological systems.[10, 11, 12] Here we report a new class of compounds with glutathione peroxidase-like properties.

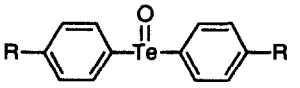
The catalytic effects of diaryl tellurides **1-11**, diaryl telluroxides **12-14** and the organoselenium compounds **15** and **16** (Figure 1) on the reduction of H_2O_2, t-BH (t-butyl hydroperoxide), CuOOH (cumene hydroperoxide) and LinOOH (linoleic acid hydroperoxide) by GSH were assessed using the coupled

reductase assay (eq. 1).[13, 14] Briefly, incubations (1 mL) were performed in 50 mM phosphate buffer at pH 7.4 containing GSH (1 mM), NADPH (250 μM) and substance (50 μM) or vehicle (DMSO, 5 μl). In incubations with organic hydroperoxides, 0.2% (w/v) Triton X-100 was included in the buffer to ensure solubility of the peroxide. The detergent did not affect either the basal or test substance-catalysed reaction of peroxide with GSH. The base-line absorption at 340 nm was recorded in a quartz cuvette. Reductase (5 μL undiluted enzyme) and peroxide (1 mM) were then added and the decline in absorbance recorded. Rate assessments were performed when the decline was constant for at least 20 seconds. In some cases up to 30% v/v DMSO was included in the incubations due to the poor solubility of some of the test compounds. Controls showed that this did not affect the reductase reaction but did accelerate the basal reduction of H_2O_2 by GSH. This was corrected for in the measurements.

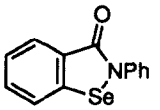
Figure 1



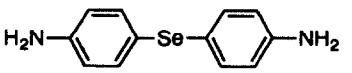
Para-substituted diaryl tellurides **1-11**



Para-substituted diaryl telluroxides **12-14**



Ebselen **15**



Bis(4-aminophenyl) selenide **16**

Of the eleven para-substituted diaryl tellurides tested, the chloro-, bromo- and nitro-substituted analogues (compounds **9-11**) were without catalytic effect on the reaction of H_2O_2 with GSH (Table 1). The most active catalysts, bis (4-aminophenyl) telluride (**1**) and bis(4-hydroxyphenyl) telluride (**2**) were three and two times, respectively, more active than Ebselen (**15**, Table 1). It will be noted that compounds **1** and **2** were the only members of the series which did not require DMSO in the buffer to ensure solubility. The remaining derivatives (compounds **3-8**) were all less active than Ebselen.

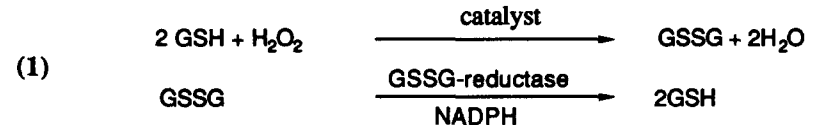


Table 1 The glutathione peroxidase-like activity of diaryl tellurides, diaryl telluroxides and reference compounds with hydrogen peroxide as substrate.

Catalyst[1]	No	%DMSO	μM/min NADPH[2]	% catalysis[3]
4,4′-Disubstituted Diaryltellurides; R=				
NH_2	**1**	0	160	2003
OH	**2**	0	112	1400
OMe	**3**	20	42	319
F	**4**	20	29	221
H	**5**	10	19	190
NMe_2	**6**	20	24	184
Me	**7**	20	19	146
CF_3	**8**	30	19	126
Cl	**9**	30	13	100
Br	**10**	30	13	100
NO_2	**11**	10	13	100
4,4′-Disubstituted Diaryltelluroxides; R=				
OMe	**12**	20	51	394
NMe_2	**13**	20	19	146
CF_3	**14**	20	11	100
Reference compounds:				
Ebselen	**15**	0	50	625
4,4′-Diamino diphenylselenide	**16**	0	8	100
Controls:				
-	-	0	8	-
-	-	10	10	-
-	-	20	13	-
-	-	30	15	-

[1] Diaryl tellurides **1**,[24] **2-5**,[23] **6**,[24] **7-11**,[23] diaryl telluroxides **12**,[25] **13**,[26] and **14**[27] and the selenide **16**[28] were prepared according to literature methods. Ebselen(**15**) was the gift of Dr M. Parnham, A Nattermann GmbH, Köln, Germany. For other materials see ref. 29.

[2] calculated over a 20 second period of stable decline of absorbance at 340 nm.

[3] calculated as: 100x(NADPH utilization + compound)/(rate of NADPH utilization - compound).

Several telluroxides (compounds **12-14**) were tested for their catalytic activities. The results with these compounds closely reflected those obtained with the corresponding tellurides (Table 1). As a comparison, the selenium analogue of compound **1**, bis (4-aminophenyl) selenide (**16**), was without catalytic effect in the test system.

Table 2 shows a comparison of the catalytic activitites of Ebselen and compound **1** on the reaction of GSH with a variety of organic hydroperoxides. The basal rates of chemical reaction between GSH and the organic hydroperoxides t-BH, CuOOH or LinOOH were 10-30% of that with H_2O_2. Ebselen was a relatively better catalyst with the organic hydroperoxides than with hydrogen peroxide. Diaryl telluride **1** showed an increasing catalytic efficiency with increasing chain-length of the peroxide, but was always more effective than Ebselen for a particular substrate. Thus, compound **1** was approximately three, five, ten and nine times as effective as Ebselen with H_2O_2, t-BH, CuOOH and LinOOH, respectively.

Table 2 A comparison of the GSH-px-like activity of Ebselen and diaryl telluride **1** with a variety of organic hydroperoxide substrates.

Compound	Substrate[1]	μM/min NADPH[2]	%Catalysis[3]	%Ebselen[4]
-	H_2O_2	15.0	-	-
-	t-BH	1.9	-	-
-	CuOOH	4.2	-	-
-	LinOOH	6.4	-	-
Ebselen	H_2O_2	75	500	-
"	t-BH	23	1210	-
"	CuOOH	35	830	-
"	LinOOH	51	797	-
1	H_2O_2	262	1740	348
"	t-BH	122	6421	530
"	CuOOH	347	8261	995
"	LinOOH	459	7403	900

[1] Incubations contained 0.2% (w/v) Triton X-100 to ensure solubilisation of the peroxides.
[2] as 2 in Table 1.
[3] as 3 in Table 1.
[4] calculated as: 100x(rate of NADPH utilisation + compound)/(rate of NADPH utilisation + Ebselen)

The results provided in this paper, demonstrating potent GSH-px-like activity of diaryl tellurides, adds one more class of compounds to the growing list of synthetic peroxidase catalysts.[13-17] The most active diaryl telluride, compound **1**, was a superior catalyst to Ebselen with all peroxides tested. The mechanism responsible for the catalysis is uncertain. In analogy with one mechanism proposed for Ebselen,[18] the initial reaction occuring in the catalytic cycle may involve oxidation of the tellurium atom to

a telluroxide. The subsequent reaction of this oxide with a thiol reducing agent would then regenerate the parent compound and complete the catalytic cycle. Diorganyl selenides and tellurides are known to be readily oxidised to the corresponding oxides by organic peroxides, as well as by hydrogen peroxide.[19, 20] These oxides are in turn useful as oxidants in the preparation of disulfides from thiols.[21, 22] Electrochemical oxidation of symmetrical diaryl tellurides revealed a strict dependence of the peak oxidation potential on the Hammett σ_p+ values of the para substituents.[23] Thus, depending on the substituents on the phenyl rings, formation of the telluroxide may occur with variable efficiency. Subsequent reaction of this oxide with GSH may also be rate-limiting and affected by electronic effects of the substituents. We recently demonstrated the extreme antioxidant capacity of compound **1** in a variety of peroxidation systems.[11] The potency of the compound could not be simply explained in terms of the redox potential or lipid solubility, and it was proposed that a telluroxide intermediate rapidly reacts with reducing agents such as ascorbate to regenerate the parent compound and establish an antioxidant cycle.[11]

The reasons for the increased effectiveness of compound **1** to catalyse the reduction of long-chain organic hydroperoxides remain obscure. Irrespective of this, the extreme effectiveness of the compound with LinOOH (see Table 2) suggests a potential in detoxifying phospholipid hydroperoxides formed in peroxidizing biological membranes.

In summary, certain *p*-substituted diaryl tellurides catalyse GSH-px-like reduction of a variety of hydroperoxide substrates. Compound **1** is one of the most potent antioxidant molecules yet described in cellular systems and a superior GSH-px-like catalyst to Ebselen, particularly with long-chain organic hydroperoxides. Thus, compound **1** should avidly interrupt lipid peroxidation at several levels in membranes subject to peroxidative insult. Organotellurium compounds may provide useful tools in the development of therapeutic agents directed towards disease states associated with lipid peroxidation.

Acknowledgements

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