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Ergothioneine – a diet-derived antioxidant with therapeutic potential

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Ergothioneine is a thiol/thione molecule synthesised only by some fungi and bacteria. Nonetheless, it is avidly taken up from the diet by humans and other animals through a transporter, OCTN1, and accumulates to high levels in certain tissues. Ergothioneine is not rapidly metabolised, or excreted in urine and is present in many, if not all, human tissues and body fluids. Ergothioneine has powerful antioxidant and cytoprotective properties *in vitro* and there is evidence that the body may concentrate it at sites of tissue injury by raising OCTN1 levels. Decreased blood and/or plasma levels of ergothioneine have been observed in some diseases, suggesting that a deficiency could be relevant to the disease onset or progression. This brief Review explores the possible roles of ergothioneine in human health and disease.

Keywords: antioxidant; ergothioneine; oxidative damage; reactive oxygen/nitrogen species

The antioxidant paradox

The generation of free radicals and related reactive oxygen (ROS),[1] nitrogen (RNS) and other reactive species[1] is an inevitable consequence of aerobic life and serves many useful purposes [1–4]. Indeed, these species were key drivers of the evolution of the huge range of aerobic organisms that we find in the world today, including ourselves [1,3,4]. Without them, we would die early from infectious disease and other disorders [1,3,5]. However, there is a downside. Certain reactive species can damage biological molecules (this is called "oxidative damage") and this oxidative damage contributes to the development of certain age-related human diseases. The evidence for a role of ROS/RNS in the origin and progression of human disease is probably strongest for certain cancers [1,6,7] and for neurodegenerative diseases such as Parkinson disease and the dementias (especially Alzheimer disease) [1,8–10], but they play roles in many other diseases as well (reviewed in [1,11]). There has, therefore, been considerable interest in developing antioxidant agents with the aim to slow the onset of, and/or to treat such diseases. Interest originally focussed (and to some extent still does) on the established diet-derived antioxidants (such as vitamins E and C) or putative ones including carotenoids and polyphenols such as the flavonoids (we say "putative" because the evidence for their antioxidant roles *in vivo* is not as strong as for vitamins E and C, as reviewed in reference [1]). However in general, the effects of diet-derived antioxidants in intervention trials on human subjects have been modest at best, and sometimes, they appear to have caused harm [1,11–15]. Multiple reasons can account for this failure, one of which is that many of these agents, administered at the high doses used in the trials, are not very effective at decreasing levels of oxidative damage in the human body [1,11,16]. There

[1]For detailed explanations of these terms please see reference [1].

Abbreviations
Aβ, amyloid beta peptide; CKD, chronic kidney disease; ET, ergothioneine; GSH, reduced glutathione; OCTN1, organic cation transporter (novel type-1); RNS, reactive nitrogen species; ROS, reactive oxygen species.

is, therefore, an ongoing search for better antioxidant agents, including synthetic ones such as NADPH oxidase inhibitors [3,17] and metalloporphyrins that can scavenge a range of ROS/RNS: some of each type are presently in clinical trials [17,18]. Another approach is to use reagents that activate the Nrf2 system, which leads to increases in the *in vivo* levels of several antioxidant enzymes and of reduced glutathione (GSH), a key cellular antioxidant [19]. Indeed, the beneficial effects (if any, the jury is still out) of polyphenols against certain diseases, such as cancer, have been suggested to relate more to their pro-oxidant abilities (raising endogenous antioxidant levels in a hormetic fashion [1,20]) than to direct antioxidant effects. However, too much Nrf2 activation may not be good [16,19,21], especially if it disturbs the normal essential cellular function of ROS/RNS.

Ergothioneine, a natural "antioxidant"?

Which brings us to ergothioneine (ET) a natural product with considerable *in vitro* antioxidant properties (reviewed in [22]). Figure 1 shows its structure; the tautomeric equilibrium favours the thione form. Studies into this thiol/thione derivative of histidine date back to the early 1900s when ET was first identified in the ergot fungus [23], hence the name. ET is synthesised by certain fungi and bacteria only, not by animals or higher plants [22,24–26]. Nevertheless, ET can be found in a wide range of foods (Table 1 shows some data from our laboratory). However, mushrooms, which are capable of ET biosynthesis along with several other fungi, are a major source in the human diet [22,25,27–29]. Interestingly, some foods have large variations in the levels of ET, for example, asparagus, which we believe to be attributed to possible symbiotic relationships of these plants with soil fungi or bacteria, or pre- or postharvest fungal contamination (Table 1). Dietary ET in animals (including humans) is absorbed by means of an intestinal transporter, OCTN1, that has a high degree of specificity [30]. The same transporter then distributes ET to most or all body tissues: excretion from the body is slow and administered ET is highly retained in human and other animal body tissues and red blood cells [22,31–33]. This implies that ET has a useful function, otherwise why absorb and retain it? By contrast, many polyphenols (which have frequently been suggested to act as important antioxidants *in vivo*) are rapidly metabolised or excreted from the body, which instead suggests that they are unwanted xenobiotics [1]. Indeed, numerous *in vitro* studies have demonstrated the abilities of ET to scavenge ROS and RNS (such as hydroxyl radicals [34], hypochlorous acid [34], singlet oxygen [35,36], and peroxynitrite [37]), modulate inflammation [38], chelate divalent metal cations such as iron and copper (thereby decreasing the ability of these metal ions to stimulate oxidative damage) [34,39,40], and protect against UV radiation-induced damage [41,42], amongst other cytoprotective activities [43]. Conversely, OCTN1 knockout animal models appear to be predisposed to oxidative stress [44,45], although more studies in rodents to characterise the phenotype in detail and verify this are needed.

An "adaptive" antioxidant?

Some studies have presented evidence that ET may be accumulated at sites of tissue injury, in particular in fatty liver disease, liver fibrosis, pressure overloaded and infarcted hearts and pre-eclampsia. This accumulation seems to relate to an increased expression of the gene encoding OCTN1, resulting in increased transporter activity [46–49]. We have suggested [46,50] that this is a deliberate cytoprotective mechanism (Fig. 2). Arguably in these situations of tissue injury, supplementation of humans with extra ET might be of therapeutic benefit [50,51]. Animal studies have suggested that ET may play a limited antioxidant role in healthy animals but may come into play when levels of reactive species rise due to tissue injury, in the presence of ROS/RNS-generating toxins, or diseases that involve increased levels of oxidative damage (reviewed in [22,50]). This concept is consistent with *in vivo* studies of the effects of dietary ET supplementation on oxidative damage in young healthy adults [32]; there was a trend to a decrease in oxidative damage, as detected in plasma and urine using several established biomarkers of oxidative damage, but no major decreases. However, this could be argued to be a useful property of ET: not interfering with the important roles of ROS/RNS in healthy tissues [1,3–5] but coming into play when oxidative damage becomes excessive due to tissue injury, toxin exposure or disease [50] and ET is

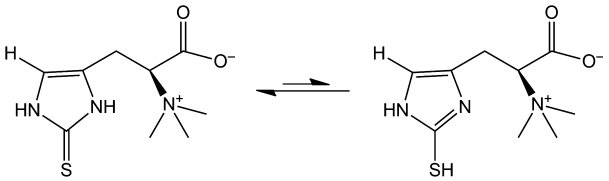

Fig. 1. The chemical structure of ET (2-mercaptohistidine trimethylbetaine; $C_9H_{15}N_3O_2S$) and its tautomers [32]. The thione form (left) predominates at physiological pH [22]. Figure reproduced from Refs [22,32].

Table 1. ET levels in a variety of mushrooms and other foods. Levels were quantified by liquid chromatography mass spectrometry (Agilent Technologies) as described in Ref.[32], using locally (Singapore) sourced foods, lyophilised and powdered. Mushrooms were kindly provided by Kok Kheng Tan (MycoBiotech). ET levels are represented as mg per 100 g dry weight in mushrooms and mg per 1 kg dry weight for all other foods, as the average of three or more samples of each food. Interestingly, some foods had large variations in ET levels depending on source, such as asparagus *, which is known in certain circumstances to grow symbiotically with fungi (mycorrhiza); asparagus itself cannot synthesise ET since its genome does not contain the necessary genes. Pre- or postharvest fungal or bacterial contamination may also alter ET levels in foods. LOQ; below limits of quantitation.

Mushrooms varieties	Ergothioneine (mg per 100 g dry weight)	Fruits and vegetables	Ergothioneine (mg per kg dry weight)
Boletus edulis (cepes)	181.24	Garlic	34.60
King Oyster	54.17	Japanese Seaweed	2.34
Buna Shimeji	43.26	Parsnip	2.23
Shiitake	35.35	Kiwi fruit	1.99
Enoki	34.64	Onion	1.13
Willow	29.68	Persimmon	1.52
Abalone	32.47	Pomegranate	1.3
White Shimeji	19.75	Passion fruit	1.22
Portobello	19.09	Durian	1.09
White button	15.44	Broccoli	0.38
Brown button	10.41	Kale	0.22
Black fungus	9.42	Tomato	0.20
Maitake	2.02	Ginger	0.17
Wood ear	0.64	Rice	LOQ
White fungus	0.58		

Nuts, beans and spices	Ergothioneine (mg per kg dry weight)	Milk and soy products	Ergothioneine (mg per kg dry weight)
Basil leaf	4.92	Tempeh	201.13
Brazilian nut	4.45	Soy beancurd	3.71
Gingko nut	3.98	Soy milk	2.31
Cumin	2.60	Fresh milk (average of 4 varieties)	0.25
Pepper	2.57	Greek yogurt	LOQ
Kidney beans	2.09		
Pistachio nut	1.90	*Asparagus varieties	
Almond	1.87	Asparagus (Malaysia)	0.57
Oats	1.84	Asparagus (Thailand)	10.24
Macadamia nut	1.65	Asparagus (Mexico)	163.25
Sweet bean	1.33	White asparagus	18.20
Ginseng root	0.69		

then accumulated, as summarised in Fig. 2. Some cells where the risk of oxidative stress is high and constant, such as erythrocytes [1], may always keep ET levels high [32,33,49,52].

Ergothioneine in tissues, extracellular fluids and cell culture

Ergothioneine can accumulate to high levels in some human and animal tissues, including red blood cells (with basal levels of ~ 125 μM and ~ 220 μM in human and mouse whole blood, respectively, and millimolar levels reported in red blood cells [52,53]), liver and spleen (with basal levels of ~ 350 $\mu mol \cdot g^{-1}$ tissue and ~ 100 $\mu mol \cdot g^{-1}$ tissue in mouse liver and spleen, respectively) [22,31–33]. Our recent study [33] demonstrated that when ET is orally administered to mice, it accumulates rapidly in the liver and blood cells but also enters most (or perhaps all) other tissues, including brain, heart, lung, kidney, spleen and eye. Since animal sera are commonly used in cell culture media, ET is even present in cell cultures. The variable ET levels in different batches and sources of sera may be a possible confounder in cell culture studies [50], given the propensity of cell culture to cause oxidative stress [20,54,55].

When ET is administered to humans, ET levels in plasma and whole blood are significantly elevated and, interestingly, continued to increase in whole blood for up to 4 weeks after administration ceased [32]. Moreover, excreted levels (in urine) were extremely low, indicating the avid retention of ET by the body [32]. We (paper in preparation) have found ET to be present in a range of other human extracellular fluids and secretions such as cerebrospinal fluid (with levels around 250 nM) and aqueous humour of the eye (with levels in the high μM range). Animal seminal fluids are reported to contain especially high levels of ET, with early studies showing that ET levels in boar seminal plasma were around six times greater than blood ET levels, and in some animals, ET is the predominant thiol/thione in seminal plasma, far exceeding levels of GSH [56–58]. Data on humans are awaited with interest.

Ergothioneine and the mother–baby axis

As a further illustration of the widespread distribution of ET and possibly the essentiality of this compound to humans (as suggested by the presence of a transporter that appears largely specific for ET [30]), we have recently identified the presence of ET in human breast milk (with ET concentrations ranging from 5 to

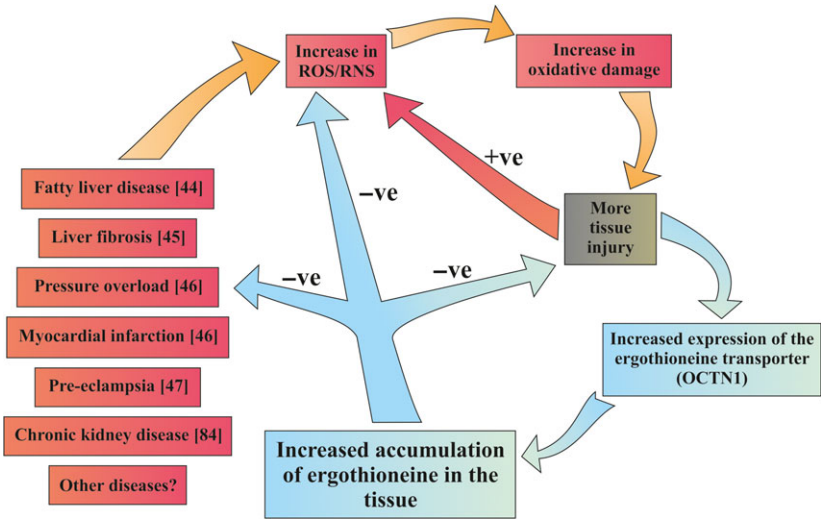

Fig. 2. Tissue injury increases ROS/RNS production *in vivo*, which can then contribute to further injury. In certain diseases or conditions, tissues may increase expression of OCTN1, leading to increased ET levels in the affected tissue. This may be a deliberate response to protect tissue by decreasing further oxidative damage [50] . +ve, increased; −ve, decreased.

150 nM; paper in preparation). ET can also be found in cow and goat's milk (with mean concentrations ~ 13 nM and ~ 9 nM, respectively) and a range of infant formulas (with a mean concentration ~ 9 nM). The latter is not unexpected, since baby formulas are typically based on milk powders. Indeed, the urine and brains of newborn babies have been reported to contain ET [59,60]. This implies that ET can cross the placenta into the baby (presumably via OCTN1, which is known to be present in placenta [61,62]) and/or that ET is absorbed from breast milk through OCTN1 in the intestines of the baby. Additionally, ET has been detected in amniotic fluid, in sheep [63]. Studies revealed that the OCTN1 mRNA expression levels in cultured human mammary epithelial cells are elevated more than sixfold during lactation, relative to nonlactating mammary epithelial cells [64]. If this happens in the intact breast, it suggests that this is a mechanism to deliver ET to the baby, from which it would follow that ET is important to the baby. However, more work is needed to establish this *in vivo*.

Ergothioneine as a potential treatment for diseases

Several studies have identified decreased levels of ET in certain tissues relative to controls, in subjects with various diseases (reviewed in [22,50], also see the sections below). This suggests potential interventions with administered ET to raise the levels as both a therapeutic and possibly a preventative agent. Studies in animals and humans have found no toxicity or adverse effects to be associated with ET administration, even at high doses, and recently, ET (Tetrahedron, Paris, France) has attained European Food Safety Authority approval in the European Union and is generally recognised as safe by the Food and Drug Administration in the US (GRAS notice 734) [65] as a supplement. Hence, the possible beneficial effects of supplementation with ET to correct these low levels are worth further investigation.

Neurodegenerative diseases

A range of *in vitro* and *in vivo* studies have demonstrated the neuroprotective capabilities of ET. Supplementation with ET dose-dependently protected rat phaeochromocytoma cells against β-amyloid (Aβ)-induced apoptotic death [66] and decreased neuronal injury caused by direct injection of Aβ into the hippocampus of mice [67]. Our own studies (paper in preparation) have demonstrated that ET can dose-dependently extend lifespan of a transgenic *Caenorhabditis elegans* model of Alzheimer disease [68], through reduction in Aβ-oligomer load. Other *in vivo* studies have also demonstrated that ET attenuates oxidative stress and prevents cognitive deficits in a D-galactose-induced mouse model of dementia [69], protects against *N*-methyl-D-aspartate-induced cytotoxicity in rat retinal neurons [70] and cisplatin-induced neuronal damage in mice [71].

In humans, plasma ET levels were found to be significantly decreased in subjects with mild cognitive impairment [72] and Parkinson disease [73] when compared to age-matched controls. This could happen by multiple mechanisms of course, such as changes in diet and/or OCTN1 transporter activity. Dietary ET does cross the blood–brain barrier, since it can be measured in human cerebrospinal fluid and postmortem brain samples (our unpublished data; also see [59]) and

readily enters the brain when administered to mice [33]. A Japanese study [74] has found a correlation between increased intake of mushrooms (one of the most important dietary sources of ET [27–29]; Table 1) and lower incidence of dementia. However, mushrooms are known to contain a wide range of possibly bioactive compounds that could account for this observation. Hence, direct studies (placebo-controlled, double-blinded intervention trials) are needed to investigate whether ET administration will have beneficial effects in neurodegenerative disorders.

Eye disorders

Early studies demonstrated that substantial levels of ET are found in the eye [75], where it was suggested to protect against chronic exposure to ROS due to high oxygen tension (the cornea is exposed to 21% oxygen), UV exposure and high metabolic activity [76]. The ocular surface, consisting of a layer of tear fluid, the cornea and the aqueous humour, forms the first line of defence against oxidative damage [1,77]. We recently identified significant levels of ET in human tear and aqueous humour samples (~ 0.35 μM and ~ 28 μM, respectively; paper in preparation), and we have demonstrated that ET readily accumulates within the eye when mice are fed with ET [33]. It is, therefore, of interest that some earlier studies revealed that significantly lower levels of ET in the lens and cornea are found in individuals with cataracts; the levels continued to decrease with increasing severity of the cataract formation [78,79]. Another study found that treatment with ET afforded modest protection against glucocorticoid-induced cataract formation in chicks [80]. No further studies have since been undertaken to follow up on this. However, if ET levels in tear fluids correlate with levels in the lens and aqueous humour, this could provide a noninvasive means of measuring eye ET levels and thereby establish the risk of developing eye disorders. Since the pathology of the major eye diseases involves oxidative damage [1,77,81], the possible protective effects of ET seem to be worthy of further investigation in various human ocular diseases.

Cardiovascular diseases

Myocardial ischaemia–reperfusion injuries are known to generate, and be exacerbated by, excessive formation of ROS/RNS, leading to oxidative myocyte damage [1,82,83]. This suggests potential benefits of antioxidants as cardioprotectants [1,82,83]. Early studies by Arduini *et al.* [84] suggested that one of the mechanisms by which ET might protect the heart is by countering the oxidation of myoglobin by ROS/RNS to the cytotoxic ferryl myoglobin, which can be a critical event in myocyte damage during cardiac ischaemia–reperfusion. The ability of ET to scavenge ROS/RNS and chelate transition metal ions may also be relevant [1,82,83]. Since administered ET readily accumulates in the blood [32] and enters the heart (at least in mice [33]), it could be useful therapeutically.

Ischaemia–reperfusion injury is not a phenomenon confined to the heart; it can occur in almost all organs including brain, liver, skin, gut, muscle and kidney [1,82]. Indeed, animal studies have shown that ET supplementation can protect several tissues, including liver [85], intestine [86] and lung (following intestinal ischaemia–reperfusion [87]), from ischaemia–reperfusion injury. Conversely, removing ET by knocking out the gene encoding the transporter in mice appears to predispose them to more injury following ischaemia–reperfusion injury [44]. Hence, ET could conceivably have therapeutic potential in these various ischaemic injuries.

Kidney disease, diabetes and cancer

A recent study describes how ET plays a role in chronic kidney disease (CKD); knockout of the gene encoding OCTN1 (which prevents ET uptake) in a mouse model of this condition worsened renal fibrosis [88]. Consistent with the relevance of this to humans, patients with CKD have lower blood ET levels [88]. Indeed, CKD is well known to involve increased oxidative damage [1,89] and ET might be helping to decrease this by accumulating in the kidney (Fig. 2). Another recent paper suggested that ET might be useful in treating or preventing pre-eclampsia [51], a condition in which oxidative damage has also been implicated as playing a significant pathological role [1,51].

Diabetes and diabetic complications are intimately linked to increased oxidative and glycative stress, in part due to the pro-oxidant effect of hyperglycaemia [1,90]. *In vitro*, ET has been observed to protect endothelial and rat pheochromocytoma (PC12) cells against the damage caused by high glucose levels [91,92], and supplementation of diabetic pregnant rats with ET decreased the occurrence of embryo malformations [93]. Earlier studies claimed that blood ET levels are elevated in diabetes [94,95], which if indeed the case (the studies should probably be repeated using modern methodologies for ET quantification) could conceivably be a secondary response to the elevation in oxidative stress (Fig. 2). Conversely, administration of the agent alloxan (which damages pancreatic β-cells

to produce a diabetic state [1]) to rabbits temporarily decreased levels of blood ET [96].

Examining the Cancer Genome Atlas database for differential mRNA expression of OCTN1 uncovered significant differences in certain cancers relative to controls. In particular, significantly increased levels of OCTN1 mRNA were seen in thyroid, liver and oesophageal cancers (our unpublished data). However, little is known about the cause of these changes in OCTN1 mRNA, and how they impact ET levels. If indeed, certain cancers do accumulate ET to help protect themselves, perhaps against chemotherapy (whose effects are known to involve oxidative stress [1,6]), then therapeutic possibilities come to mind, such as depleting ET and/or blocking the transporter.

Conclusion and possible caveats

ET appears to be a safe, natural diet-derived antioxidant whose therapeutic potential looks promising but remains to be validated by the gold standard of double-blind, sufficiently powered, human clinical trials. One caution is that not only certain fungi but also some microorganisms make ET and use it to protect themselves against attack by host defences (including ROS/RNS): as evidenced by the fact that inactivation of their capacity to make ET decreases their virulence [97,98]. One of the most prominent of the ET-synthesising microorganisms is the pathogen, *Mycobacterium tuberculosis*, the causative agent of human tuberculosis [24,97,98]. Indeed, inhibitors of the ET biosynthetic pathway (which should be a feasible therapeutic approach since this pathway does not occur in humans) could be a potential source of novel drugs to attack this and other pathogenic ET-synthesising organisms, such as that causing melioidosis [99]. One possibility is that *M. tuberculosis* not only makes ET but can also take it up from the environment (we have found that several bacteria can do this; our unpublished data); if the injury to the infected lung was to raise ET content in lung tissue (Fig. 2), this could be one example (not uncommon in the antioxidant world [1]) where a mechanism that is normally cytoprotective ends up making things worse. Similarly, in cancer (Section 5.4), the elevation in OCTN1 mRNA in certain cancers could lead to increased ET levels in the cancer, which might help to protect it against chemotherapeutic agents, as mentioned above.

Another issue is the mechanism by which ET can protect cells and tissues. From the properties of this molecule *in vitro*, antioxidant effects seem likely. However, other protective mechanisms may exist [99,100], and more work is required to elucidate them.

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Author contributions

BH provided the initial framework and wrote most of the Review. IKC assisted in writing and revising the Review. IKC and RMYT provided experimental data used in the Review.

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