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Minireview

Hydrogen peroxide in the human body

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Abstract Hydrogen peroxide (H_2O_2) is widely regarded as a cytotoxic agent whose levels must be minimized by the action of antioxidant defence enzymes. In fact, H_2O_2 is poorly reactive in the absence of transition metal ions. Exposure of certain human tissues to H_2O_2 may be greater than is commonly supposed: substantial amounts of H_2O_2 can be present in beverages commonly drunk (especially instant coffee), in freshly voided human urine, and in exhaled air. Levels of H_2O_2 in the human body may be controlled not only by catabolism but also by excretion, and H_2O_2 could play a role in the regulation of renal function and as an antibacterial agent in the urine. Urinary H_2O_2 levels are influenced by diet, but under certain conditions might be a valuable biomarker of 'oxidative stress'.

Key words: Hydrogen peroxide; Hydroxyl radical; Urine; Breath; Catalase; Oxygen electrode

1. Introduction

Hydrogen peroxide (H_2O_2) is a pale-blue covalent liquid, freely miscible with water and apparently able to cross cell membranes readily, although the pathways it uses to traverse have not been elucidated [1]. Multiple papers have described high (usually ≥50 μM) levels of H_2O_2 as being cytotoxic to a wide range of animal, plant and bacterial cells in culture, although LD_{50} values and the mode of cell death induced (apoptosis or necrosis) depend on the cell type used, its physiological state, length of exposure to H_2O_2, the H_2O_2 concentration used, and the cell culture media employed [1–5]. It is therefore widely thought that H_2O_2 is very toxic in vivo and must be rapidly eliminated, employing enzymes such as catalases, peroxidases (especially glutathione peroxidases) and thioredoxin-linked systems [1,6–9]. Paradoxically, however, acatalasemia in humans [1] appears to produce no significant phenotype, nor does 'knockout' of glutathione peroxidase in mice except under certain conditions of abnormally high oxidative stress [10–13].

In chemical terms, H_2O_2 is poorly reactive: it can act as a mild oxidizing or as a mild reducing agent, but it does not oxidize most biological molecules readily, including lipids, DNA and proteins (unless the latter have hyper-reactive thiol groups or methionine residues [1,3,14]). The danger of H_2O_2 largely comes from its ready conversion to the indiscriminately reactive hydroxyl radical ($OH^\bullet$), either by exposure to ultraviolet light [15]

$$H_2O_2 \xrightarrow{uv} 2OH^\bullet$$

or by interaction with a range of transition metal ions, of which the most important in vivo is probably iron [1,16]

$$Fe^{2+} + H_2O_2 \rightarrow \text{intermediate complexes (ferryl??)} \rightarrow$$

$$Fe^{3+} + OH^\bullet + OH^-$$

Living organisms have evolved mechanisms to sequester transition metal ions into protein-bound forms that cannot catalyze $OH^\bullet$ formation and other free radical reactions in vivo. These mechanisms are especially important in such extracellular fluids as the blood plasma [1,16,17]. Nevertheless, H_2O_2 can contribute to Fenton chemistry not only by being one of the substrates but also by providing the other, e.g. by liberating iron from heme proteins [1,16–19]. Addition of H_2O_2 to cells in culture can lead to transition metal ion-dependent $OH^\bullet$-mediated oxidative DNA damage, although this damage appears to be rapidly repaired provided that the cells are not rendered non-viable by an excess of H_2O_2 [20].

However, levels of H_2O_2 at or below about 20–50 μM seem to have limited cytotoxicity to many cell types. Indeed, there is a growing literature showing that H_2O_2 can be used as an inter- and intra-cellular signalling molecule [21–26]. The first example to be elucidated was the role of H_2O_2 as a second messenger in the activation of NFκB in some [23], but not all [27], cell types. Other examples of signalling roles for H_2O_2 have accumulated fast [21–26]. Hence these may be a good reason not to eliminate all the H_2O_2 generated in vivo; its use in physiological signalling mechanisms. At sites of inflammation, H_2O_2 generated by activated phagocytes appears to modulate the inflammatory process, e.g. by up-regulating expression of adhesion molecules, controlling cell proliferation or apoptosis and modulating platelet aggregation [3,4,28–33].

2. Exposure of human tissues to H_2O_2

Hydrogen peroxide is generated in vivo by the dismutation of superoxide radical ($O_2^{\bullet-}$), both non-enzymatically and catalyzed by superoxide dismutase enzymes. Hydrogen peroxide is also directly produced by a range of oxidase enzymes in-

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cluding glycollate and monoamine oxidases as well as by the peroxisomal pathway for β-oxidation of fatty acids [1,6,34,35]. Transgenic mice lacking mitochondrial superoxide dismutase suffer severe pulmonary and neurological damage, indicating the essentiality of removing intra-mitochondrial $O_2^{\bullet-}$ in vivo [36–38]. However, with the apparent exception of cardiac muscle, mitochondria in most tissues appear to have limited capacity to remove H_2O_2, in that they readily generate substantial amounts of H_2O_2 in vitro and probably in vivo [1,6,35,39–41]. Although mitochondria contain glutathione peroxidase and thioredoxin-linked peroxidase activities [42–44], the efficiency of these enzymes in removing H_2O_2 is uncertain given the ease with which mitochondria release H_2O_2 [1,6,39–41].

It thus seems likely that most or all human cells are exposed to some level of H_2O_2, with the mitochondria being an important source. However, certain tissues may be exposed to higher H_2O_2 concentrations.

2.1. The oral cavity, oesophagus and stomach

Several beverages commonly drunk by humans can contain H_2O_2 at concentrations above 100 μM, including green and black tea and especially instant coffee [45–47]. When such beverages are ingested, the H_2O_2 they contain presumably rapidly diffuses into the cells of the oral cavity and upper part of the gastrointestinal tract [48]. Oral bacteria also produce H_2O_2 [49,50], although the resulting levels of exposure of the oral tissues are uncertain. It is often suggested that H_2O_2 released into saliva is used by salivary peroxidase to oxidize thiocyanate (CNS^-) into products toxic to certain bacterial strains [50].

2.2. The respiratory system

The cells lining the respiratory system, in common with the oral and oesophageal epithelium, are exposed to high O_2 concentrations (21%) as compared with most other body tissues [1]. Hydrogen peroxide is present in exhaled air of humans [51–59] and of rats [60], although it is uncertain whether this H_2O_2 originates from oral bacteria [49,50], phagocytes (e.g. alveolar macrophages, neutrophils in the oral cavity, or neutrophils recruited to the lungs in inflammatory lung diseases) or other lung cells. Amounts of exhaled H_2O_2 appear greater in subjects with inflammatory lung diseases [52–58] and in cigarette smokers [59]. Nevertheless, H_2O_2 is present in the air exhaled by healthy human subjects [51–59].

Table 1
Levels of hydrogen peroxide in freshly voided human urine

Gender of subject	Age (years)	$[H_2O_2]$ in urine (μM)
Female	18	5.0
Female	19	8.0
Female	19	0.4
Female	21	6.2
Female	22	7.7
Female	25	11.5
Female	27	13.0
Female	35	3.5
Male	20	26.5
Male	21	16.3
Male	23	5.2
Male	26	5.9
Male	28	18.9
Male	30	22.3
Male	34	11.0
Male	49	109.6

Spot urine samples were collected from healthy human volunteers and assayed immediately. Subjects undertook no special dietary or other preparation before providing samples. Data are means of replicate determinations on each sample; replicates varied by $<5\%$. H_2O_2 was analyzed by the ferrous ion oxidation–xylenol orange assay. Some data abstracted from [63], the rest provided by Caroline Manonmani, Mangala Srinivas, Melissa Sim and Yogeshwar Emritoll, students enrolled in the Talent Development Programme of the National University of Singapore.

2.3. The kidney, urinary tract and bladder

Substantial quantities of H_2O_2, at concentrations sometimes exceeding 100 μM, can be detected in freshly voided human urine (Table 1) [61–63], even in babies [64]. The simplest way of demonstrating its presence is to place urine into an oxygen electrode, and inject catalase through the cap. A 'spike' of O_2 release results as the H_2O_2 present is decomposed by catalase [63].

The H_2O_2 detected in human urine appears to arise, at least in part, by $O_2^{\bullet-}$-dependent autoxidation of urinary molecules, some of which originate from diet [47,63,65]. Traces of superoxide dismutase are present in urine [66]: this enzyme, as well as the acidic pH of urine, should facilitate both enzymic and non-enzymic dismutation of $O_2^{\bullet-}$ to H_2O_2 [1]. The pO_2 of urine within the bladder is below that of ambient air [67,68] and so the rate of H_2O_2 generation in urine may well increase upon voiding. Nevertheless, the high levels of H_2O_2 that can be detected in some urine samples (Table 1) strongly suggest that at least some H_2O_2 generation occurs within the bladder. Indeed, H_2O_2 has been detected in urine sampled by catheterization [69]. Hydrogen peroxide has an antibacterial effect [1,2,70] and it may be that its presence at high levels in urine could be advantageous in diminishing infections of the bladder and urinary tract. On the other hand, the impact of H_2O_2 generation in vivo upon the cells lining the kidney tubules, ureters, bladder and urinary tract must be considered. Indeed, there are suggestions that H_2O_2 is involved in modulation of renal function [71–73]. Another possibility is that excretion of H_2O_2 represents a metabolic mechanism for controlling its levels in the human body. If so, measurement of urinary H_2O_2 levels may represent a valuable tool for assessment of 'oxidative stress', since H_2O_2 can be measured rapidly and simply [63,65]. This suggested route of H_2O_2 elimination by excretion is perhaps analogous to certain fish, which appear to dispose of H_2O_2 by excreting it through their gills [74].

2.4. Vascular endothelial and circulating blood cells

Some studies have claimed substantial levels of H_2O_2 (up to $\sim$35 μM) in human blood plasma [75–77], but others have claimed levels to be very low, at or close to zero [78]. The latter data seem more credible, since H_2O_2 added to human plasma disappears rapidly. In part, it is degraded by the traces of catalase present, but H_2O_2 can also react with heme proteins, ascorbate, and protein-SH groups [1,79]. In vivo, H_2O_2 generated in plasma could also diffuse into erythrocytes, white cells, endothelial cells and platelets for metabolism. However, the studies in [75–77] could be interpreted to suggest that H_2O_2 can be detected at high levels in plasma under assay conditions in which its removal is prevented. This implies that human plasma may be continuously generating H_2O_2. One enzyme involved in this process, at least under pathological conditions, appears to be xanthine oxidase [80]. Levels of

circulating and endothelium-bound xanthine oxidase are increased as a result of tissue injury [81,82].

2.5. Ocular tissues

The presence of H_2O_2, at widely varying levels (in some cases, 100 μM or more), has been reported in human and other animal aqueous and vitreous humors [83,84]. The explanation might be essentially the same as that advanced above to account for the conflicting data reported for blood plasma, i.e. that ocular fluids constantly generate H_2O_2, which is rapidly removed [83]. Any impairment in the capacity of the lens epithelium, retina or other ocular tissues to dispose of H_2O_2 would then result in its accumulation. The origin of this H_2O_2 is uncertain, but oxidation of glutathione or ascorbate is one possibility [84].

3. Conclusion

Hydrogen peroxide appears to be a ubiquitous molecule. We exhale it, excrete it and take it in from diet. It can be detected in drinking water, rain water and sea water [85–89]. These data emphasize the importance of metal ion sequestration in preventing the toxicity of H_2O_2 in vivo by decreasing the occurrence of Fenton chemistry, and help explain why a failure of such sequestration can produce devastating tissue damage in almost all organs of the body [1,16].

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