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Review

Systematic review of ophthalmate as a novel biomarker of hepatic glutathione depletion

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SUMMARY

Background: Sustainability of hepatic glutathione (GSH) homeostasis is an important cellular defense against oxidative stress. Therefore, knowledge of liver GSH status is important. However, measurement of plasma GSH and tissue is difficult due to its instability. Alternatively, ophthalmate (OPH), an endogenous tripeptide analog of GSH, has been suggested as a potential indicator to assess GSH depletion.
Aim: To provide an overview of present knowledge with respect to the usefulness of OPH as a biomarker for oxidative stress and hepatic GSH homeostasis.
Methods: A systematic, computerized search combined with a cross-reference search of the literature described in PubMed (January 1975 to January 2012) was conducted, key words: 'ophthalmate' and 'ophthalmic acid'.
Results: Twenty-two articles were included. Hepatic OPH levels increase inversely proportional to a drop in hepatic GSH in mice with paracetamol (PCM) induced hepatotoxicity. Little is known about the stability of OPH in human plasma. To measure the very low physiological concentrations of plasma OPH, liquid chromatography-mass spectrometry techniques can be employed. OPH synthesis can be measured in humans, using stable isotope labeling with a deuterated water (^2H_2O) load.
Conclusion: OPH may be a promising biomarker to indicate hepatic glutathione depletion, but the suggested biological pathways need further unraveling.

1. Introduction

The anti-oxidant glutathione (GSH) is the main hepatic protection system against redox imbalances and many forms of oxidative stress and intoxications.[1–3] Unfortunately, thus far assessment of hepatic GSH homeostasis in vivo in humans has remained to be difficult, yet the availability of an adequate test to measure hepatic GSH depletion would be of great clinical importance. In order to estimate the GSH pool of the liver a liver biopsy would be required, which necessitates an invasive procedure. Even when such a liver biopsy can be obtained, measurement of the GSH content of a liver biopsy alone does not provide information on the capacity of the liver for de novo GSH synthesis. Attempts have been made to measure hepatic GSH depletion in vivo in human plasma, but this proved to be difficult because of instability of GSH due to auto-oxidation.

Recent research has shown that ophthalmate (OPH), which is an endogenous analog of GSH, can be used as a potential marker for GSH depletion.[4–6] OPH is synthesized when GSH is depleted and when the hepatic availability of cysteine is limited. OPH is tripeptide very similar to GSH (glutamate–glycine–cysteine) but in OPH the cysteine moiety is replaced by the non-protein amino acid 2-aminobutyrate. OPH is synthesized through the same enzymatic machinery as GSH, but OPH lacks the reducing cysteine moiety and therefore it is far more stable than GSH.[6] Since OPH is released into the blood stream, a rise in plasma OPH concentration may thus indicate hepatic GSH depletion. In this context, plasma OPH has been suggested to be a reciprocal read out of hepatic intracellular GSH content. This review gives an overview on the state of the art knowledge on the potential of OPH to be used as a biomarker for oxidative stress and hepatic intracellular glutathione depletion.

Abbrevations: GSH, reduced glutathione; GSSG, glutathione disulfide (oxidized glutathione); GCS, glutamyl-cysteine-synthetase; GS, glutathione-synthetase; OPH, ophthalmate; 2AB, 2-aminobutyrate; ROS, reactive oxygen species; NAPQI, N-acetyl-p-benzoquinone imine; LC–MS, liquid chromatography tandem mass spectrometry.

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2. Methods

2.1. Literature search strategy

A systematic, computerized search of the literature described in PubMed (January 1975 to January 2012) was undertaken independently by two investigators (SD and EN) using a combination of the key words 'ophthalmate' and 'ophthalmic acid'. All obtained abstracts were read and relevant studies were selected using three levels of screening. The first exclusion criterion was language other than English. The second exclusion criterion involved absence of relevance to hepatic metabolism, such as ophthalmology. Finally the full text of every study was considered. If there was a discrepancy in inclusion between the two investigators, a decision was reached by a third reviewer (CD). Finally, papers obtained via cross-referencing were also included.

3. Results

Forty papers were identified from PubMed. In total 26 papers were excluded for review: in 2 papers the language was other than English and 24 papers involved OPH in relation to other topics mostly ophthalmology and were not relevant to hepatic metabolism (Fig. 1). Of these forty papers, fourteen papers fell within the scope of this review. Three papers described OPH as a marker for glutathione depletion[5–7] and 2 papers described technique of measuring OPH.[8,9] Two papers investigated the relation between the GSH/OPH redox buffer system in primary and secondary liver tumors.[4,10] Three papers studied the breakdown of OPH and its physiological function.[11–13] Finally 4 papers investigated the synthesis of OPH and GSH.[14–17] Eight additional papers were identified by manual cross-referencing and were included: 5 general papers on GSH metabolism,[18–22] 1 paper on paracetamol (PCM) hepatotoxicity[1] and 2 papers on OPH synthesis.[23,24] Thus, 22 papers were considered appropriate for inclusion for review.

3.1. Glutathione

Many forms of stress, sepsis, ischemia and toxic compounds are associated with severe redox imbalance in cells. Glutathione (GSH) is the main intracellular antioxidant and thus protects cells against oxidative stress caused by radicals from reactive oxygen species (ROS). These radicals are produced continuously through intracellular processes in mitochondria, by enzyme actions of cytochrome P-450 (CYP450) and exposure to environmental factors such as ultraviolet-radiation.

The CYP450 system is one of the main metabolic pathways of generation of ROS.[18] Radicals damage DNA, lead to protein oxidation and cell dysfunction and ultimately cell death.[19] Therefore scavenging radicals is important for maintaining cell integrity and function. Apart from its antioxidant capacities, GSH also seems to play a role in modulation of cell proliferation, immune responses and cell signaling.[20] As the main site of drug transformation, the liver is particularly exposed to radicals.

The antioxidant properties of GSH are dependent on a sulfide group in the cysteine moiety. GSH can scavenge radicals in two different ways: (1) by forming a dimer through a disulfide bridge, whilst capturing a radical in this bond and forming the oxidized GSSG (glutathione disulfide), and (2) by conjugation to reactive molecules. Glutathione peroxidase (GP) catalyzes the formation of GSSG. The dimer can be reduced to the original GSH state by GSSG reductase, using NADPH as a reductant agent. Glutathione S-transferase (GST) catalyzes the conjugation of GSH to the reactive molecules.

3.2. Synthesis of glutathione

The liver is the major GSH storage organ and the major source of plasma GSH. GSH is a tripeptide consisting of the amino acids glutamate, cysteine and glycine. It is synthesized by the sequential actions of γ-glutamylcysteinesynthetase (GCS) and GSH synthetase (GS). First, GCS binds cysteine to γ-glutamate, producing γ-glutamylcysteine. Then GS binds glycine to γ-glutamylcysteine producing γGlu–Cys–Gly (glutathione). GSH exerts a negative feedback on GCS, limiting its own synthesis if sufficient GSH is present. The rate-limiting enzyme in this cascade is GCS while GS is probably not a rate-limiting step.[21] GSH synthesis depends on the availability of cysteine (Fig. 2). Cysteine is synthesized in the liver from methionine through homocysteine via the transsulfuration pathway. Therefore, the liver is not only the major GSH storage organ, but also the major source of plasma GSH. GSH is released across both the canalicular membrane into bile and across the sinusoidal membrane into blood for delivery to other tissues.[22]

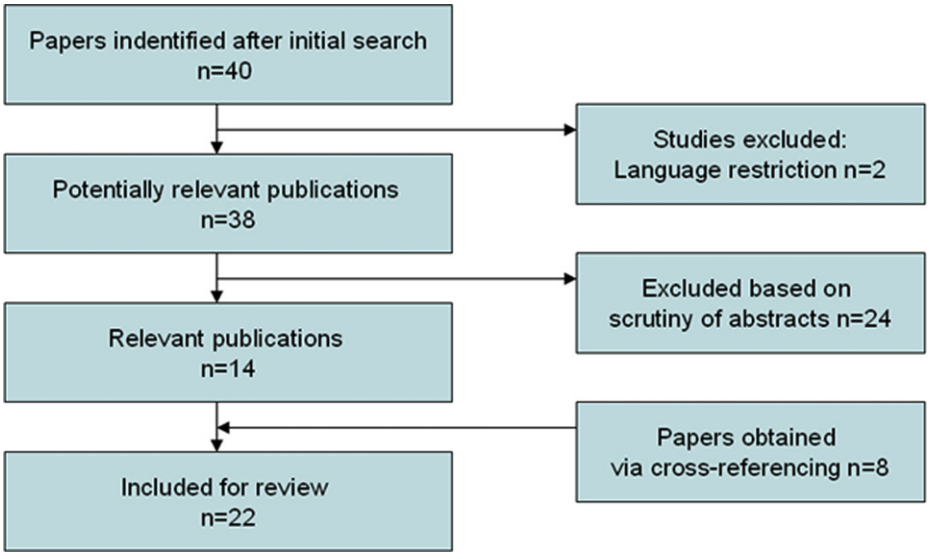

Fig. 1. Selection of articles for review.