Free Radical Research, June 2009; 43(6): 533–537

informa
healthcare

Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants?

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(Received 25 November 2008; revised 11 March 2009)

Abstract
It was previously hypothesized that serum gamma-glutamyltransferase (GGT) within its reference range predicts various clinical outcomes as a sensitive marker of oxidative stress in humans. This study further hypothesizes that serum GGT can mark exposure to various environmental pollutants, based both on recent epidemiological findings and on well-established biochemical features of cellular GGT. Cellular GGT is a prerequisite for metabolism of GSH conjugates that detoxify xenobiotics to mercapturic acid. Under this concept, serum GGT may increase with increasing exposure to environmental pollutants which need to be conjugated to GSH. Supporting this concept, it was recently reported that serum GGT within its reference range was linearly associated with important environmental pollutants, including lead, cadmium, dioxin and organochlorine pesticides. As a marker of the amount of conjugated xenobiotics, recent epidemiological findings about serum GGT imply the possibility of harmful effects of various environmental pollutants at background levels currently regarded as safe.

Keywords: *Gamma glutamyltransferase, glutathione, xenobiotics, oxidative stress, environmental exposure*

Introduction

Recent epidemiological studies have demonstrated that serum gamma-glutamyltransferase (GGT) predicted various important clinical outcomes including diabetes, myocardial infarction and cancer [1–3]. In particular, most associations showed dose–response relationships within the reference range of serum GGT in the general population. Although abnormal serum GGT (above its reference range) has traditionally been used as a marker of alcohol consumption [4] or hepatobiliary disease [5], findings in non-drinkers as well as in drinkers and dose–response relations observed within GGT's reference range lead us to conclude that neither alcohol abuse nor hepatobiliary problems explain why GGT predicts chronic disease. Although it is unclear what mechanisms are involved in the associations between serum GGT and these diseases, which have diverse pathological mechanisms, we initially considered that the involvement of GGT in oxidative stress might be important [6].

Serum GGT as a marker of oxidative stress

GGT exists on the cell membrane with its catalytic site GGT directed into the extracellular space [7]. Although GGT is expressed in most tissues, higher activity of GGT is found in kidney tubules, biliary epithelium and brain capillaries [8]. GGT is involved in cellular homeostasis of glutathione (GSH), the major intracellular antioxidant tripeptide [9]. GGT metabolizes extracellular GSH to provide component amino acids for intracellular GSH resynthesis in a continuous GGT-mediated 'GSH cycle' [7].

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ISSN 1071-5762 print/ISSN 1029-2470 online
DOI: 10.1080/10715760902893324

Therefore, GGT has traditionally been regarded as a major factor in the reconstitution of cellular antioxidant defenses. The role of cellular GGT in intracellular GSH homeostasis led us to hypothesize that serum GGT predict various diseases as a marker of oxidative stress [6].

As oxidative stress is regarded as a key pathogenetic element in various diseases [10], at first glance this interpretation on serum GGT as a marker of oxidative stress appeared to be reasonable. However, a difficulty with this concept is that elevated serum GGT activity should decrease the risk of disease because elevated cellular GGT activity leads to the increased synthesis of intracellular GSH, contrary to the epidemiological findings that serum GGT is most often positively related to disease risk.

Another prevailing interpretation in which serum GGT is regarded as a marker of oxidative stress holds that GGT is directly involved in generating reactive oxygen species (ROS) [11]. This view is based on experimental findings in which cysteinylglycine, one of the products of GGT action on GSH, has a strong ability to reduce Fe^{3+} to Fe^{2+}, which again promotes generation of ROS with high reactivity [12]. Recent pathology studies [13,14] have suggested the independent role of GGT in the pathogenesis of cardiovascular diseases brought by atherosclerosis: its activity has been observed in coronary atherosclerotic plaques, colocalized with low density lipoprotein (LDL) and foam cells [13]. Moreover, it has been shown that glutathione hydrolysis by GGT can trigger iron-catalysed LDL oxidation [14], as well as production of reactive oxygen species [14], likely promoting plaque complications. For this reaction to occur *in-vivo*, however, the presence of redox-active iron is a prerequisite [15]. In fact, under the presence of redox-active iron, other common antioxidants such as ascorbic acid can also generate ROS with high reactivity, such as the hydroxyl radical [16]. However, the availability of redox-active iron is tightly regulated under normal physiological situations [16]. While there are many examples in human pathology in which 'unusual' physiologic mechanisms that are chronically maintained can act in a detrimental way, pro-oxidant effects of GGT seem unlikely when serum GGT is at low normal-to-moderate levels, yet the main epidemiological findings on serum GGT do observe dose–response risk gradients from low normal to moderate levels in the general population. Furthermore, a pro-oxidant effect of GGT should be more prominent in the elderly than in the young, since serum GGT tends to increase with age and body iron stores are great in the elderly. However, epidemiological studies observed weak or no predictability of serum GGT in old persons [1,2]. Therefore, even though a pro-oxidant role for serum GGT could be contributory to some epidemiological findings, consideration of alternative pathways linked to certain physiological functions of GGT may offer additional insight.

Pollutants and serum GGT

We recently reported in a representative sample of the US general population that serum GGT within its reference range was linearly associated with internal dose of several important environmental pollutants, including lead, cadmium, dioxin and organochlorine pesticides [17,18]. Importantly, these associations were observed across their whole ranges of these pollutants in a representative sample of the US general population. In contrast, serum alanine aminotransferase (ALT), another liver enzyme that is associated with serum GGT in the general population, did not show positive associations with these pollutants, suggesting a unique feature of serum GGT. In fact, as ALT is contained in cytosol and mitochondria, not cell membrane [19], elevation of serum ALT is not observed unless hepatocytonecrosis or increased permeability of hepatocellular membrane is induced. This suggests that background levels of pollutants which are not sufficient to induce hepatocyte damage can increase serum GGT through unknown mechanisms. These findings led us to hypothesize that the associations between serum GGT and various diseases may indirectly reflect harmful effects of low levels of environmental pollutants.

How does serum GGT increase with the exposure to pollutants?

Detoxification denotes a large set of reactions that typically lower the toxicity and increase the water solubility of a wide range of endogenous and xenobiotic compounds, including environmental pollutants [20]. Traditionally, these reactions have been grouped in phases. Phase I entails the introduction of a functional group, often by cytochrome P450 catalysed hydroxylation, that may either decrease or increase toxicity. In Phase II, the newly introduced or a pre-existing functional group is conjugated with molecules such as glucuronic acid, sulphate or GSH to reduce toxicity and increase solubility. Phase III involves transport of the conjugate out of the cell.

GSH plays a major role in detoxifying many reactive metabolites by either spontaneous conjugation or by a reaction catalysed by the GSH S-transferases [21]. GSH S-transferases have broad and overlapping substrate specificities, which allow them to participate in the detoxification of a chemically diverse group of compounds [21]. The most common reactions involve nucleophilic attack by GSH on electrophilic carbon: saturated carbon atoms (e.g. alkyl halides, lactones and epoxides), unsaturated carbon atoms (e.g. α, β-unsaturated compounds, quinones and

quinoniamines and esters) or aromatic carbon atoms (e.g. aryl halides and aryl nitro compounds) [21]. These substrates have in common a degree of hydrophobicity and possess electrophilic centres [21].

GSH also forms metal complexes via non-enzymatic reactions [22]. GSH is one of the most versatile and pervasive metal binding ligands and plays an important role in metal transport, storage and metabolism [22]. The sulphydryl group of the cysteine moiety of GSH has a high affinity for metals, forming thermodynamically stable but kinetically labile mercaptides with several metals, including cadmium and lead [22].

Just as cellular GGT is indispensable for metabolism of extracellular GSH, GGT is also needed to prepare extracellular GSH conjugates for transport out of the cell and further metabolism to mercapturic acid, the final form of GSH conjugates found in urine or bile [23]. In this way, higher serum GGT plausibly reflects increased cellular GGT activity involved in metabolizing extracellular GSH conjugates. Thus, serum GGT may increase with increasing exposure to environmental pollutants which need to be conjugated to GSH.

In addition to exogenous electrophilic xenobiotics, however, endogenous compounds such as byproducts of lipid peroxidation are also conjugated by GSH [24]. Thus, serum GGT can be seen as a general marker of a variety of compounds which are conjugated by GSH. Depending on the situation, compounds which mainly increase serum GGT may be different; for example, serum GGT in persons exposed to various pollutants may primarily reflect the exposure to pollutants. Thus, the strong secular trend of serum GGT observed in South Korea may reflect a fairly sudden increase in exposure to various pollutants [25]. On the other hand, serum GGT among persons with substantial oxidative stress may primarily reflect the increased production of endogenous compounds such as byproducts of lipid peroxidation. For example, the association between obesity and serum GGT [26] may reflect the increased production of endogenous compounds in relation to oxidative stress due to adiposity [27]. These two conditions, exposure to exogenous electrophilic xenobiotics and endogenous generation of lipid peroxides, are not mutually exclusive and may reinforce each other because the exposure to pollutants itself increase oxidative stress [28].

Serum GGT as a marker of compounds which need to be conjugated to GSH is also helpful in explaining why serum GGT failed to predict future risk of cardiovascular diseases or cancer among older persons [1,2]. This may be related to a decreasing ability to clear xenobiotics with ageing, similar to other physiological functions [29]. Cellular and therefore serum GGT increase proportionate to the amount of glutathione conjugates, not to the amount of the compounds themselves that ought to be conjugated to GSH. Thus, we can expect lower production of glutathione conjugates per unit compound exposure in older vs younger persons.

Our viewpoint on serum GGT as a marker of environmental pollutants can be seen as contradictory to findings in the Australian Twin Registry that serum GGT has high heritability [30]. Metabolism of xenobiotics is greatly influenced by many enzymes of phase I and phase II reactions [31] and polymorphisms of genes coding these enzymes are very common. Under our interpretation, GSH conjugates are produced in response to chemical exposure to induce GGT. The amount of GSH conjugates may be influenced by both the amount of environmental pollutants and the action of enzymes of the phase I and phase II reactions. Thus, in this mechanism that involves many genes with common polymorphisms, it would be expected that serum GGT show heritability in twins. In addition, GGT could show some heritability in twins due to shared foetal environment. The exposure to environmental pollutants starts *in utero* because many chemicals transfer across the placenta [32]. There is emerging evidence that epigenetic changes can occur due to environmental exposure to chemicals *in utero* [33].

Pollutants may explain some associations between serum GGT and diseases

It is important to note that serum GGT increased at environmental pollutant levels that are presumed to be safe in the general population [17,18]. People are daily, simultaneously exposed to very low levels of several hundreds or thousands of different xenobiotics, well below the no observable adverse effects levels (NOAELs) of the individual compounds. Thus, epidemiological findings linking serum GGT within its reference range to various clinical outcomes may be evidence of the harmful effects of very low levels of environmental pollutants. However, we think that this risk may not reflect harmfulness of a very low level of any individual pollutant, but be due to mixed effects of various environmental pollutants. Although experimental studies observed significant mixture effects from multiple exposures with low dose of NOAELs [34], it is very difficult to confirm these effects in humans. Thus, the prediction of important clinical outcomes by serum GGT provides human evidence of harmfulness of exposure to very low levels of mixed xenobiotics, if, as we hypothesize, the level of GGT reflects background exposure to environmental pollutants.

In fact, this hypothesis led us to focus on Persistent Organic Pollutants (POPs), persistent lipophilic xenobiotics that accumulate in adipose tissue [35]. Similar to serum GGT, we found that serum concentrations

of POPs were associated with various clinical outcomes in the general population [36–40], suggesting that some associations of serum GGT may be explained by POPs. In particular, epidemiological findings linking POPs to type 2 diabetes, including strong dose–response relations and an interaction between POPs and obesity on the risk of type 2 diabetes, closely resembled the same associations of diabetes with serum GGT [35,40]. Our hypothesis is that GGT level reflects exposure to a mixture of xenobiotics; in parallel, even studies of a specific POP in the general population may reflect mixed effects of several hundred POPs, because many POPs in the general population have common exposure through food consumption.

Increased serum GGT may also reflect chronic GSH depletion

The associations between serum GGT and disease outcomes may not be explained solely by direct effects of environmental pollutants. The consumption of GSH through GSH conjugation due to chronic exposure to environmental pollutants can lead to the depletion of intracellular GSH [9]. GSH protects cells from oxidative stress by reacting with hydrogen peroxide, superoxide anion, singlet oxygen and hydroxyl radical or by participating as a substrate to GSH peroxidase activity, which catalyses the elimination of hydrogen peroxide, lipid peroxides and peroxynitrite [9]. Moreover, GSH can help in the redox cycling of antioxidants such as ascorbate and α-tocopherol [9].

Therefore, inhibition of GSH synthesis or depletion of GSH alters the balance between pro- and antioxidant molecules, amplifying the effects of ROS produced by cellular metabolic activity. GSH has recently been implicated in protection against the induction of apoptotic and necrotic cell death in a variety of cell types [41]. Thus, it is not surprising that an imbalance of GSH is observed in a wide range of pathologies, including cancer, cardiovascular diseases, neurodegenerative disorders and ageing [42]. Some associations between serum GGT and diseases may be caused by chronic depletion of intracellular GSH, without any involvement of pollutant themselves. Furthermore, direct toxicity of environmental pollutants can be also exaggerated under the condition of GSH depletion [43].

Conclusion

Recently, the strong predictability of various important diseases by serum GGT has been a topic of scientific discussion. Although the mechanisms linking GGT to disease need further clarification, GGT can be used in clinical settings as a cheap and easy risk assessment. However, weak predictability of serum GGT in older persons may limit its clinical usefulness, because most diseases occur in old persons.

Based on well-known biochemistry of cellular GGT and epidemiological studies of serum GGT, we propose that recent epidemiological findings about serum GGT imply the possibility of harmful effects of background exposure to various environmental pollutants, even though those background levels are currently regarded as safe. Harm may arise from direct toxicity of the mixed pollutants themselves, indirect toxicity through GSH depletion or in combination.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 16 April 2009.