

Unbalanced biotransformation metabolism and oxidative stress status: implications for deficient fatty acid oxidation

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ABSTRACT

The concept of accumulating xenobiotics within the human body as a health risk is well known. However, these compounds can also be endogenous, as in the case of inborn errors of metabolism, and lead to some of the same symptoms as seen in xenobiotic intoxication. Biotransformation of both exogenous and endogenous toxic compounds is an important function of the liver, and the critical balance between these systems is of fundamental importance for cellular health. We propose a novel model, to describe the critical balance between Phase I and Phase II biotransformation and how a disturbance in this balance will increase the oxidative stress status, with resulting pathological consequences. We further used deficient fatty acid oxidation to verify the proposed model, as deficient fatty acid oxidation is associated with the accumulation of characteristic metabolites. These accumulating metabolites undergo both Phase I and Phase II biotransformation reactions, with resulting depletion of biotransformation substrates and co-factors. Depletion of these important biomolecules is capable of disturbing the balance between Phase I and Phase II reactions, and disturbance of this balance will increase oxidative stress status. The value of the proposed model is illustrated by its application to a clinical case investigated in our laboratory. In this case the possibility of deficient fatty acid oxidation only became evident once the critical balance between Phase I and Phase II biotransformation was restored with oral replenishment of biotransformation substrates. In addition to biochemical improvement, there was also significant clinical improvement. The significance of this model lies within the treatment possibilities, as the assessment of

biotransformation metabolism and oxidative stress status can lead to the development of nutritional treatment strategies to correct imbalances. This in turn may reduce the chances of, or delay the onset of certain disease states.

Keywords: Biotransformation Metabolism; Detoxification; Fatty Acid Oxidation; Oxidative Stress Status

1. INTRODUCTION

The indispensable role of the liver in the biotransformation or detoxification of a variety of exogenous and endogenous compounds is accomplished by two groups of enzymatic modifications known as Phase I and Phase II biotransformation metabolism. Phase I reactions expose functional groups to form reactive sites, which improve water solubility of the compound itself, or allow Phase II reactions to ensue when the products of Phase I biotransformation are conjugated with endogenous hydrophilic compounds to enhance their excretion [1-3]. However, during Phase I functionalization the resultant reactive molecule can in certain cases be more toxic than the parent compound, and effective neutralization of these noxious compounds is important in preventing covalent binding of the reactive metabolites to proteins, lipids and nucleic acids [2,3].

Maintaining the balance between Phase I and Phase II reactions is therefore of paramount importance, and under normal circumstances these enzymes function adequately to minimize inefficient detoxification and potential induced intracellular damage. However, an overloaded or unbalanced system negatively affects the oxidative stress status, with serious health compromising consequences [3,4].

The metabolic processes that are fundamental for maintaining normal cell structure and function are highly regulated enzyme catalyzed processes. Defects in these enzyme systems, whether induced or inherited, have

significant consequences in man, *i.e.* the accumulation of toxic substrates upstream of the enzyme defect, disturbances in metabolic intermediates downstream of the enzyme defect, and the formation of intermediates by alternative biochemical pathways [5]. On a clinical level these biochemical aberrations will give rise to various pathological conditions including acute life-threatening encephalopathy, hyperammonemia, metabolic acidosis, hypoglycemia, jaundice and liver dysfunction [6]. This can ultimately lead to the development of chronic diseases and eventual death.

Biotransformation metabolism is a well studied discipline within the pharmaceutical industry, and the concept of accumulating xenobiotics within the human body as a health risk is well known. However, the accumulation of endogenous compounds in the case of inborn errors of metabolism and its pathological consequences is typically not explicitly associated with unbalanced biotransformation metabolism.

Explaining the development of the phenotypic characteristics of metabolic diseases is a formidable challenge. To this end we propose a model to help explain the pathological outcomes of induced and inborn errors of metabolism. This model entails that unbalanced biotransformation metabolism due to depletion of Phase II substrates and co-factors can be the first linkage in a chain of events with severe pathological outcomes. It is vital for scientific advancement and clinical applications that the phenomenon of unbalanced biotransformation metabolism be considered as a primary cause of metabolic aberrations manifesting as increased oxidative stress status. The proposed unbalanced biotransformation metabolism model will be illustrated using defective β -oxidation of fatty acids, and its value will be demonstrated by its application in the development of individualized treatment protocols for patients suffering from induced and/or inborn errors of metabolism.

2. THE UNBALANCED BIOTRANSFORMATION METABOLISM MODEL

In the unbalanced biotransformation metabolism model, a hypothesis is proposed to describe the critical balance between Phase I and Phase II biotransformation and how a disturbance in this balance will increase the oxidative stress status, with resulting pathological consequences. A defect in, or inhibition of any one of the many enzymes involved in cellular metabolism results in the accumulation of specific metabolites that need to be removed from the body either via alternative pathways or by Phase I and Phase II biotransformation metabolism. Phase I biotransformation of accumulating metabolites

and alternative pathways, both result in additional formation of reactive oxygen species (ROS). Induced Phase I biotransformation will furthermore increase the burden on Phase II conjugation and the increased demand on the latter could lead to the depletion of conjugation substrates and co-factors. Depletion of these biomolecules will disturb the critical balance between Phase I and Phase II biotransformation, which will further increase the oxidative stress status, ultimately leading to the depletion of the endogenous antioxidant capability, further affecting Phase II conjugation. Increased circulating ROS will cause oxidative damage to macromolecules such as lipids, proteins, and nucleic acids, and some of these adducts will contribute to the depletion of endogenous antioxidants. If these reactive adducts are not neutralized effectively they can diffuse to different sites and intensify the effects of oxidative damage by decreasing respiratory chain activity. This model therefore proposes that unbalanced biotransformation metabolism form an additional “vicious cycle” for increased oxidative stress status which originates from inefficient biotransformation.

3. REGULATION OF THE CRITICAL BALANCE BETWEEN PHASE I AND PHASE II BIOTRANSFORMATION METABOLISM

Biotransformation metabolism is under homeostatic regulation to control the detoxification of xenobiotics and their metabolites. This homeostatic system includes both negative feedback control as well as feedforward processes. In Phase I negative feedback control, xenobiotics activate a range of receptors to induce Phase I enzymes [7]. In most cases Phase I activity prepares the arena for Phase II conjugation to take place, because the Phase I intermediate metabolites activate transcription factors to induce synthesis of Phase II conjugation enzymes, also by means of negative feedback control [2,3]. However, many Phase II enzymes are also upregulated directly by the parent xenobiotic, which entails feedforward control by the reactive metabolites formed during Phase I. This reduces the response time for the biotransformation system to adapt and remove harmful Phase I intermediates more rapidly. However, there are also other factors involved in this process, such as nutrient concentration control [7]. Phase I biotransformation requires little nutritional support, whereas Phase II requires various co-factors and substrates, which must be replenished by dietary sources [2,3]. Therefore, although biotransformation metabolism is under homeostatic regulation which includes both negative feedback and feedforward control, depletion of Phase II substrates and co-factors

will undeniably disrupt the critical balance between Phase I and Phase II biotransformation.

4. CONSEQUENCES OF DISTURBED BALANCE IN BIOTRANSFORMATION METABOLISM

The main intracellular source of ROS is the mitochondrial respiratory chain. However, some enzymes including NADPH oxidases and cytochrome P450-dependent oxygenases also produce ROS during their enzymatic reactions [8]. ROS normally exist in all aerobic cells in balance with tightly controlled antioxidant defence and repair mechanisms. A steady state of oxidative stress, which is always present in cells, can therefore increase (increased oxidative stress status) if the endogenous antioxidant system is not capable of coping with the continuous ROS production, or if an uncontrolled increased ROS production occurs [9].

One of the most important endogenous antioxidant molecules is reduced glutathione (GSH), as it plays an important role in neutralizing free radicals. A shift in the ratio between reduced glutathione (GSH) and oxidized glutathione (GSSG) could therefore further increase the oxidative stress status. In addition to its antioxidant function, GSH is also involved in Phase II conjugation, which can occur spontaneously or in an enzyme reaction catalyzed by glutathione-S-transferases (GSTs) [10,11].

Compromised biotransformation can also have a great influence on the content and type of fatty acids and steroids involved in cellular signaling. Increased circulating ROS and free fatty acids cause lipid peroxidation and the formation of aldehyde by-products, including 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). Detoxification of these lipid peroxidation by-products enhances glutathione depletion even further. If these reactive molecules are not neutralized they can diffuse to different sites and intensify the effects of oxidative stress by decreasing respiratory chain activity [12,13].

5. VERIFICATION OF THE UNBALANCED BIOTRANSFORMATION METABOLISM MODEL: DEFICIENT FATTY ACID OXIDATION

At least 25 enzymes and transport proteins, various co-factors, co-enzymes, and substrates such as L-carnitine, co-enzyme A, FAD and NAD are involved in mitochondrial β -oxidation, and genetic defects in at least 22 of these proteins cause disease in humans [14-16]. In addition to inborn errors in fatty acid oxidation, various xenobiotic compounds can also lead to inhibited enzyme activ-

ities, e.g. Aspirin (acetylsalicylic acid), a widely used analgesic, and Valproate (VPA), a branched-chain fatty acid, which is clinically used in the treatment of various seizure disorders. Acetylsalicylic acid is rapidly hydrolyzed to salicylic acid upon ingestion, and is then activated to salicyl-CoA before conjugation to glycine can take place. VPA, on the other hand, undergoes the same metabolic reactions as natural fatty acids, including mitochondrial β -oxidation, peroxisomal β -oxidation, and cytochrome P450 dependent ω - and ω -1 hydroxylation [17].

Deficient mitochondrial fatty acid oxidation results in the accumulation of free fatty acids and acyl-CoA species [14,17]. These metabolites need to be removed from the body either via alternative pathways, or biotransformation metabolism (Phase I and Phase II) (**Figure 1**). The alternative pathway to mitochondrial β -oxidation occurs in the peroxisomes. The first step in this pathway is catalyzed by acyl-CoA oxidase, which involves the reduction of oxygen to hydrogen peroxide [18-20]. Phase I biotransformation of accumulated fatty acids involve cytochrome P450 dependent ω -oxidation of fatty acids [21,22]. During fatty acid ω -oxidation the corresponding dicarboxylic acids of the metabolized fatty acids are formed [22]. In addition, ROS is also formed during this reaction via flavoprotein mediated donation of electrons to molecular oxygen [23] (**Figure 1**). Both the alternative pathway and Phase I biotransformation metabolism can therefore result in enhanced production of ROS.

Phase II biotransformation of accumulated acyl-CoA and Phase I generated dicarboxylic acids involve conjugation with either glycine or L-carnitine [14-16]. Subjects with deficient fatty acid oxidation will therefore present biochemically with elevated levels of carnitine and glycine conjugates of acyl-CoA and dicarboxylic acid species.

The increased demand on Phase II biotransformation to maintain the critical balance can result in the depletion of these Phase II conjugation substrates (**Figure 1**). If these substrates are not replenished, the critical balance between Phase I and Phase II biotransformation will become disturbed. When this balance is disturbed due to sustained induced Phase I biotransformation and reduced Phase II conjugation, it could increase the oxidative stress status [3] (**Figure 1**), with a consequent shift in the GSH:GSSG ratio, that could exacerbate the oxidative stress status and affect Phase II conjugation [10,11].

An increased amount of circulating ROS molecules, in addition to accumulated free fatty acids, especially poly-unsaturated fatty acids (PUFAs), can further worsen this condition, as ROS could attack these fatty acids and initiate lipid peroxidation. Lipid peroxidation results in the formation of aldehyde by-products, in-

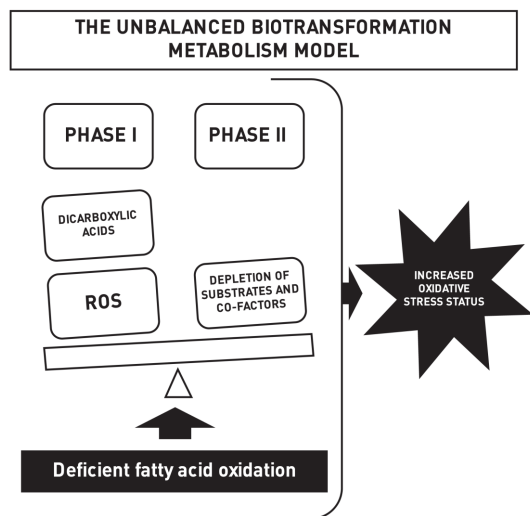


Figure 1. Disturbance in the critical balance between Phase I and Phase II biotransformation metabolism by deficient fatty acid oxidation can ultimately lead to an increased oxidative stress status, which is the underlying mechanism for the development of various pathologies.

cluding 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) [12,13]. Increased presence and distribution of these peroxidized lipid metabolites could furthermore lead to mitochondrial instability, as phospholipids are an indispensable constituent in mitochondrial membranes for the functional assembly of the respiratory chain. The incorporation of these lipid derivatives into mitochondria could therefore lead to decreased respiratory chain activity, with resulting increased oxidative stress status [13].

Moreover, it has recently been demonstrated that two of the accumulating free fatty acids in MCAD deficiency (octanoate and decanoate) lead to increased oxidative stress status [24], and the uncoupling of oxidative phosphorylation [25] in rat brain tissue. Unbalanced biotransformation metabolism and the consequent increase in oxidative stress status are therefore a possible cause in the development of certain neurological consequences in these kinds of deficiencies.

In addition to an increased oxidative stress status, the disturbed biotransformation balance can also generate the pathological condition known as co-enzyme A (CoA) sequestration, toxicity and redistribution (CASTOR) [26]. This phenomenon has been demonstrated in both inborn fatty acid oxidation deficiencies and xenobiotic induced fatty acid oxidation deficiencies [17,26]. The accumulation of acyl-CoA intermediates will lead to decreased availability of free CoA and acetyl-CoA molecules, and changes in these levels can disrupt various metabolic pathways. These metabolic pathways include the Krebs cycle, ureagenesis, biotransformation path-

ways as well as the mitochondrial redox state. It could also lead to further deficiencies in downstream products within these metabolic pathways [26]. Taken together, defective fatty acid oxidation and its concomitant biochemical characteristics clearly verify the proposed unbalanced biotransformation metabolism model.

6. IN VIVO APPLICATION OF THE UNBALANCED BIOTRANSFORMATION METABOLISM MODEL

The value of the proposed model is illustrated by its application to a clinical case investigated in our laboratory. A non-smoking female Caucasian, 57 years of age presented with chronic fatigue, coughing, dyspnoea, pain and anorexia and was diagnosed with metastatic small cell carcinoma of the lung. The cancer also metastasized to the liver although liver function tests were within the reference range. After the diagnosis she was started on a chemo combination therapy, called CAV, which consists of Cyclophosphamide, Doxorubicin and Vincristine for six repeated cycles over a period of twenty weeks. For the whole assessment time she continued with a prescribed medication regimen consisting of: Epilim (sodium valproate), Lamictol (Lamotrigine), Leponex (Clozapine) and Simvastatin (Simvastatin, ascorbic acid and butylated hydroxyanisole).

Four weeks before the end of chemotherapy, the subject suffered from severe fatigue and the first biotransformation and oxidative stress status assessments were done. This assessment was performed by challenging Phase I and Phase II biotransformation reactions with appropriate probe substrates. Caffeine was used as a probe substrate for CYP1A2 activity (Phase I), and paracetamol and aspirin as probe substrates for glucuronide conjugation, sulfate conjugation, glutathione conjugation and glycine conjugation (Phase II) [3]. In addition to this, the total acylcarnitine profile and oxidative stress status parameters including the ferric reducing antioxidant power (FRAP assay), the ROS assay, measurement of hydroxyl radical markers like catechol and 2,3-dihydroxybenzoic acid (2,3-DHBA) as well as the determination of total glutathione were also included in this assessment.

From the results obtained during the initial assessment, it was evident that the biotransformation metabolism and antioxidant defense systems of this subject were functioning below normal. The activity of Phase I (CYP1A2) measured as the caffeine clearance value, as well as all the measured end products for the different Phase II conjugation reactions were also in the lower part of the reference range, with glycine conjugation being very low. The measured concentration of free carnitine was just within the reference range. The total glutathione (GSH

and GSSG) concentration was low, with ROS levels and 2,3-DHBA levels being exceptionally high.

The results of this initial assessment were used to develop an individualized nutritional supplementation protocol in which various compounds that can be divided into different classes including antioxidants, mitochondrial support supplementation and biotransformation substrates and co-factors were employed. After the introduction of this individualized nutritional treatment strategy, several follow-up investigations were performed over a period of 7 months to monitor both biochemical and clinical characteristics.

Shortly after the introduction of the nutritional supplementation treatment, the Phase I activity was markedly elevated, which could lead to the formation of more free radicals. However, after a few weeks the Phase I activity stabilized at levels well within the reference range. All the Phase II reactions also improved, with considerable improvement in glucuronide, sulfate and glutathione conjugation. Although the glycine conjugation also improved, values remained just below or just within the reference range. In addition to this the total available glutathione and the serum FRAP also increased with concomitant decreased ROS and 2,3-DHBA concentrations. The amount of free carnitine increased substantially after only eight weeks of starting the supplementation regimen. However, the ratio between acylcarnitines and free carnitine was slightly elevated. After careful investigation of the total acylcarnitine profile, the source of the elevated ratio between acylcarnitines and free carnitine in these assessments was due to increased levels of medium-chain acylcarnitines and medium-chain dicarboxylcarnitines, including hexanoylcarnitine, octanoylcarnitine, adipylcarnitine and suberylcarnitine.

It is evident in this case that the biotransformation and antioxidant defense systems were initially markedly compromised. The identification of the accumulated metabolites usually seen in fatty acid oxidation deficiencies is the most significant observation in this regard. Initial concentrations of Phase II substrates were so depleted that these metabolites were only observed after oral replenishment of the main conjugation substrate. Once the critical balance between Phase I and Phase II biotransformation was restored, the oxidative stress status decreased to levels within the reference range. In addition to the biochemical improvement, the subject also showed a significant clinical improvement, and although these results are only preliminary, it supports the value of the proposed unbalanced biotransformation metabolism model.

7. CONCLUSION

The significance in testing this model lies within the treatment possibilities, not only for inborn errors of fatty acid metabolism, but also for induced fatty acid oxidation deficiencies. It can furthermore also be significant in various metabolic aberrations manifesting as increased oxidative stress status. If the disturbance in this critical balance is indeed the first link in a chain of reactions to follow, which ultimately lead to pathological conditions like cancer, the assessment of these reactions is of immense importance. This kind of assessment can lead to the development of individualized treatment protocols to replenish important substrates and co-factors needed for the safe elimination of accumulated toxic compounds.

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