

BIOTRANSFORMATION: CAN WE APPLY THIS TO ALLERGY AND IMMUNOLOGY?

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ABSTRACT

During medical training all doctors are taught the importance of the cytochrome P450 (CYP) system and the detoxification functions of the liver. An important challenge for pharmaceutical companies is launching new pharmaceutical entities that have an acceptable safety profile and this usually entails finding molecules with a limited effect on the CYP system. Biotransformation seems to be a novel concept in allergy and immunology but ironically it has been used extensively in the medical field over many decades. This article is aimed at providing a differential diagnosis to those patients that do not quite fit the allergic picture/profile, using the concept of biotransformation to analyse them and ideally find a new solution to their disease profile. Examples of these extraordinary patients include those with numerous drug reactions but with no common 'allergic' trigger, recurring upper airway infections or chronic otitis media and common variable immunodeficiency, difficult to control asthmatics with concomitant inflammatory diseases like ulcerative colitis and the 'allergic' rhinitic with no obvious causative allergen.

INTRODUCTION

Biotransformation is the liver's ability to convert toxic substances to less toxic metabolites. During biotransformation hydrophobic molecules are converted to water soluble conjugates which can then be more easily excreted in bile and urine.

Biotransformation consists of three phases. Phase one involves enzyme activity consisting of oxidation, reduction and hydrolysis, and converts substrates into more polar molecules. The CYP is one of the major players in this phase of biotransformation.

In Phase two, metabolites with more functional groups are conjugated by glutathione (GSH), sulphate, glucuronate or glutamate. This leads to more soluble metabolites, which increases the excretion rate.

In Phase three, efflux proteins are important in determining the bioavailability of certain xenobiotics (foreign molecules), drugs, toxins and bioactive food components. In many scenarios the phases follow each other sequentially, but often metabolites will be excreted after phase one, and only

certain metabolites will undergo phase two detoxification. Secondary to the up- or down-regulation of the different enzymes within the different phases, the concept of drug-drug and drug-nutrient interactions may be explained by the process of biotransformation.¹

During the different phases, not only do enzymes play a functional role, there are additional co-factors contributing to efficacy of the detoxification process. As an example, GSH is a co-factor for the phase three protein efflux system, p-glycoprotein. Once a substrate reaches the intestinal mucosa the efflux protein, together with the co-factor GSH, is responsible for efflux of a portion of the drug into the lumen of the gut, thus affecting the bioavailability of drugs, toxins and food. Inducing phase three efflux can explain drug resistance in chemotherapy.¹ An important question that would arise from this process is: 'might this mechanism explain steroid resistant asthma or even eczema?'

Biotransformation can be influenced by the host genotype, environmental factors and may even vary from day to day within the same individual.

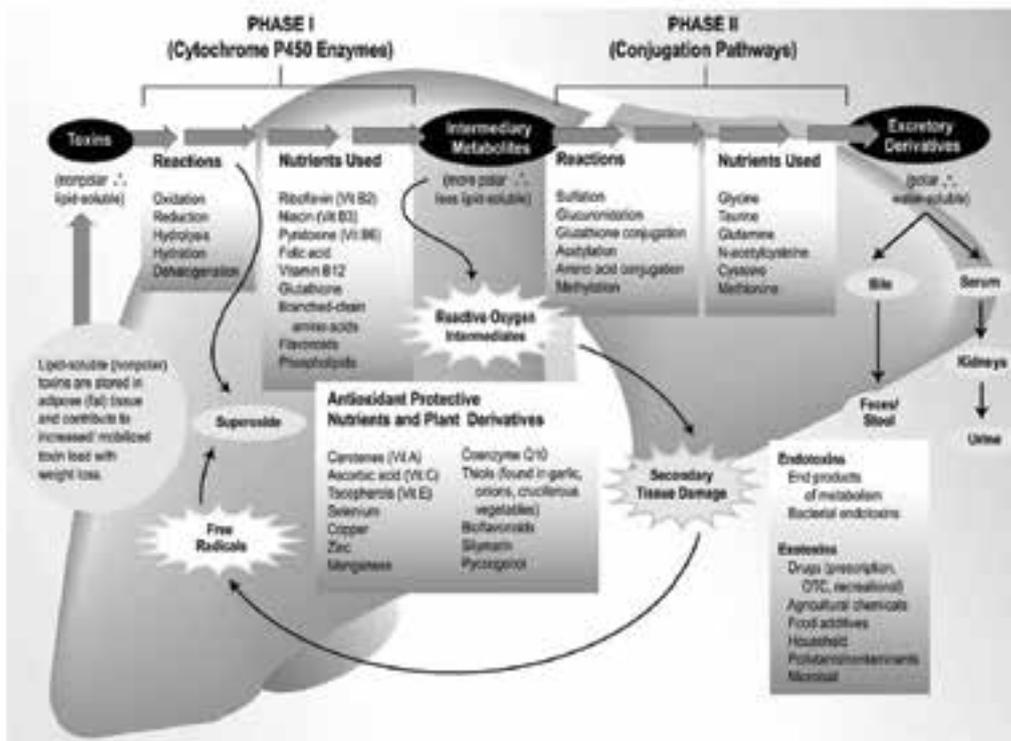


Figure 1. Liver detoxification pathways and supportive nutrients

Figure 1: An illustration of the enzymatic processes with Phase 1 and 2*2

LINKING BIOTRANSFORMATION TO PRO-INFLAMMATORY PROCESSES

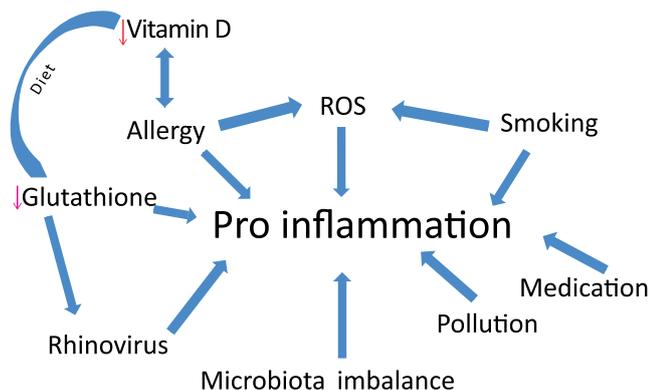


Figure 2. Linking biotransformation to pro-inflammatory processes

ROS - reactive oxygen species

DIET

Although the benefit of vitamin D supplementation in the management of allergy has revealed conflicting results, there may be certain patients where supplementation

of vitamin D in the diet may provide benefit to improve 'allergic' symptoms. Here it might be postulated that a combination of numerous co-factors may be beneficial in the management of a pro-inflammatory condition and not just the use of a single co-factor.

Vitamin D3 enhances redox balance in two ways. Firstly, it suppresses oxidative stress and secondly, it suppresses reductive stress. Oxidative stress is suppressed by reducing the iron-dependent lipid oxidation of cell membranes, increasing the activity of gamma-glutamyl transpeptidase, enhancing the synthesis of reduced glutathione and inducing manganese dependent superoxide dismutase and metallothioneins. Reductive stress is suppressed by enhancing glucose-6-phosphate dehydrogenase activity and lowering glutathione reductase activity. All of these processes subsequently lead to a reduction in free radical formation and, in turn, protect against depletion of GSH stores.³

Jain and Micinski demonstrated a positive link between vitamin D and glutathione levels, and that some beneficial effects of vitamin D supplementation may be mediated by an improvement in the cellular GSH levels and a decrease in reactive oxygen species (ROS) and pro-inflammatory cytokines.⁴

GLUTATHIONE

As previously mentioned, GSH is an important co-factor in phase two and phase three. With reactive substance formation, during CYP oxidation, the GSH pool is exhausted at a rate that exceeds replacement. This imbalance in the GSH pool allows the reactive oxidation product to bind to cellular components, including proteins, lipids, and DNA, thereby resulting in free radical production, secondary to cell death.

Spielberg *et al* demonstrated the symbiotic relationship between GSH and the protection offered to neutrophils whilst releasing ROS.⁵ Ghezzi has, in addition, demonstrated that GSH not only plays a role in the anti-inflammatory mechanisms in the lung, but also plays a role in the migratory effect of polymorphonuclear neutrophils (PMN) away from the lung and towards the site of possible infection.⁶

The GSH stores in the liver can be depleted within a day, if the host fasts or starves. In general, the whole body has about a four day reserve of GSH, but due to normal homeostasis, GSH may be supplemented from muscle or other tissue.

Increasing the intake of GSH through dietary means is neither an easy, nor practical task. An increased intake of methionine can increase the production of cysteine. Cysteine together with glycine and glutamate can lead to an increased synthesis of GSH. Fitzpatrick *et al*, suggested the liver content of GSH is similar to the daily human allowance.⁷ Such daily allowances are 1.1 g/day for a 60-kg woman (equal to 2.7 g/day of GSH) and 1.4 g/day for a 75-kg adult male (equal to 3.3 g/day of GSH), for the sulphur amino acids (methionine plus cysteine).⁸

All of these additional co-factors can be supplemented in the diet, however, the most valuable tool is that measurement, through biotransformation assays, is now possible, ensuring far better control of these co-factors and the administration of dietary supplementation. Foods containing glycine include fish, beans or legumes, meats and dairy products. Garlic contains s-allyl cysteine which is a derivative of cysteine. Asparagus contains the highest amount of glutathione but must be eaten raw. Not only is it difficult to increase the intake of these foods on a daily basis, but supplementation to increase the body stores during illness, is even more difficult.

REACTIVE OXYGEN SPECIES AND POLLUTION/SMOKING/ALLERGENS

ROS is a free radical produced through numerous metabolic processes in the body, however, overproduction of ROS can lead to mitochondrial, lipid, protein and nucleic acid damage, which in turn may produce inflammation and cell damage.

Oxidants may be inhaled, in the form of cigarette smoke and pollution, and in addition may be released from activated neutrophils, alveolar macrophages, eosinophils, and epithelial cells.

In the asthmatic airway there is a burst release of ROS in the lungs with exposure to allergens, gaseous pollutants, bacteria and viruses. Pulmonary ROS can lead to activation of pro-inflammatory cytokines and chemokine genes leading to an increase in pro-inflammatory mediators.⁹⁻¹²

MacPherson *et al* demonstrated that reactive nitrogen species can play an important role in the oxidative damage to proteins in asthmatic subjects and in addition, demonstrated that in severe asthma, eosinophils may generate NO-derived oxidants. This, in turn, may lead to increased oxidative stress and subsequently, to inactivation of super oxide dismutase, an enzyme important for down regulation of ROS.¹³

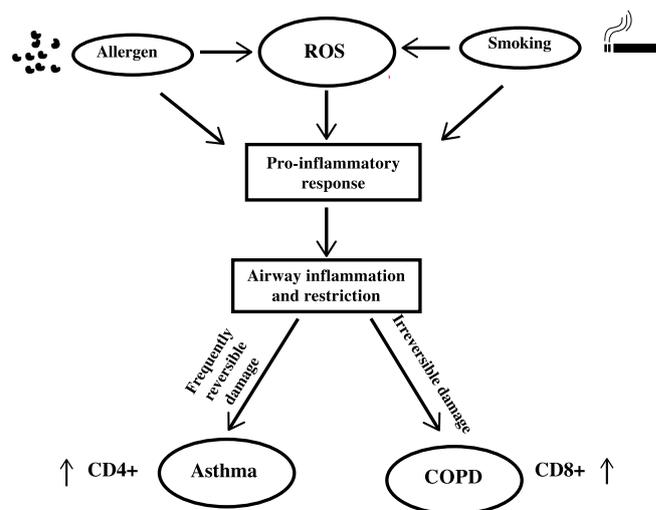


Figure 3. Proposed redox activation mechanism in asthma and COPD³

VIRUSES AND PRO-INFLAMMATORY PROCESSES

The rhinovirus is the most common cause of exacerbations of asthma. It is a well-known fact that the rhinovirus infects the respiratory epithelium, leading to inflammatory cytokine release and secondary inflammation in the airways. Intracellular adhesion molecule-1 acts as a receptor for human rhinovirus, and atopic asthmatics have an increase in intracellular adhesion molecule-1 receptors, increasing susceptibility to rhinovirus infections.

Papi *et al* have demonstrated that the rhinovirus induces oxidative stress secondary to the production of O₂⁻. O₂⁻ production is secondary to the activation of xanthine dehydrogenase/xanthine oxidase (XD/XO) thus leading to depletion of GSH which, in turn, provides further activation

of the XD/XO enzyme system.¹⁴ Papi *et al* were able to show that by enhancing the intracellular GSH storage, rhinovirus infections were unable to induce O₂⁻ production.¹⁴

Casola *et al* demonstrated that the respiratory syncytial virus (RSV) induces ROS and, in addition, may induce chemokine synthesis leading to intense pulmonary inflammation. Hosakote *et al* employed an antioxidant mimetic to reveal a decrease in RSV induced chemokines and cytokines.^{15,16} An important question that arises from these studies is: 'With viral induced/persistent wheezing, is the recurrent wheeze not possibly secondary to GSH depletion and can this not explain why some children "outgrow" their asthma and others not?'

MEDICATION AND PRO-INFLAMMATORY PROCESSES

As previously discussed, the biotransformation phases can take place in numerous areas of the body, including the mitochondria. Once a mitochondriopathy is in process, oxidative stress becomes a problem and energy production, in the form of ATP, suffers. Some of the xenobiotics that can cause fatty acid oxidation disorders in the mitochondria are tetracycline derivatives, non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and naproxen, glucocorticoids, antidepressants such as amitriptyline and nortriptyline, some statins, fibrates, oestrogens, and some antiarrhythmics and antianginal drugs, such as amiodarone and perhexiline. These drugs are in common use in the modern patient and age and, of concern, is the use of NSAIDs syrups for children.¹⁷

CONCLUSION

Although numerous questions may arise regarding the relevance of biotransformation in allergy and immunology, it remains an exciting new concept that requires exploration. Biotransformation testing is now possible and, using these results, may permit supplementation of co-factors in the management of such patients. An important message here is to carefully select these patients and to remember that by adding supplementary co-factors does not necessarily provide a cure for pro-inflammatory conditions. However, there is evidence now that there may be some help for the potentially corticosteroid resistant asthmatic, the adult with a late onset immunodeficiency and recurring upper respiratory infections, and even the multiple drug 'allergic' patients with no common denominator. There is a long way to go in exploring these very exciting conditions. Our patients deserve this condition to be fully explored.

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