Original Article

Generation of Reactive Oxygen Species and its Defense Mechanism during Aflatoxin B_1 Contamination: An Overview

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Abstract

Aflatoxins (AFs), the poly-substituted bi-furanocoumarins, are the secondary metabolites of parasiticus/flavus group of Aspergillus genus. AFs including aflatoxin B_1 (AFB_1) have been classified under Class I Human Carcinogen by the International Agency for Research on Cancer (IARC) based on its potential hepatotoxic, nephrotoxic, mutagenic, genotoxic and immunotoxic nature. Cell injury caused by AFB_1 are associated with consequences of the lipid peroxidation due to the release of free radicals and thereby damaging the biological systems. It is evident that since all the cell membranes are highly rich in polyunsaturated fatty acids (PUFAs) that could act as the substrates for such reactions. The most vital among such reactions is the AFB_1-induced oxidative stress and its consequent generation og reactive oxygen species (ROS) and the oxidative DNA damage. Normally AFB metabolites produced under phase 1 metabolism would pass through the Phase 2 metabolism via., glutathione-S-transferase (GST) enzymatic processes allowing further detoxification and elimination of the toxin from the human system. This review is mainly to focus the role of antioxidants in metabolizing the AFB_1 and thereby preventing the free radical induced peroxidation to prevent the predisposition of normal hepatocytes into malignant.

Key word: Aflatoxin B_1 (AFB_1); Free radicals; Lipid peroxidation; Antioxidants; Phase 1 & 2 metabolism

INTRODUCTION

Aflatoxins (AFs) are the most ample fungal toxins that are abundantly found the foodstuffs. Initially it was isolated from the *Aspergillus flavus* and thereby taken the name AF. Since then there are at least 20 different types of AF intermediates been identified from *Aspergillus* species (1, 2). Basically the chemical structure of AF is named as furanocoumarins. In general, AFs are most commonly found in cereals and cereal-based food that includes the rice, maize, sorghum, millets, groundnuts, dried cassava, and many others those have been stored without proper processing. Despite contaminating the food stuff, AF could also be found in the edible tissues, milk, and eggs after the consumption of feed contaminated with AFs by the farm animals (1, 3). Among the various types of AFs, aflatoxin B_1 (AFB_1) is one of the most toxic, mutagenic, and carcinogenic to both humans and livestock, and therefore, the International Agency for Research on Cancer has classified AFs as group I carcinogen (4). Consumption of AFB_1-contaminated feed could severely causes growth rate reduction, body and organ weights decrease (5), lowered egg production and re-productivity (6), immunosuppression (7) and great susceptibility to diseases (8). Before all these consequences, the basic and foremost ailments are the generation of free radicals and lipid peroxidation.

Free radicals and lipid peroxidation

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital (9). The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants or reductants. The most important free radicals in many diseases including cancer are oxygen derivatives, particularly superoxide ($O_2^{\cdot-}$) and the hydroxyl radical ($OH^{\cdot}$). Radical formation in the body occurs by several mechanisms involving both endogenous and environmental factors (Figure 1).

Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred as "Pro-Oxidants". On the other hand, compounds and reactions disposing of these species, scavenge them by, suppressing their formation, or opposing their actions are "antioxidants". In normal cell, there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted toward the pro-oxidants when production of oxygen species is increased greatly (eg., following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (eg., by inactivation of enzymes involved in disposal of oxygen species). This state is called "Oxidative Stress" and can result in serious cell damage if the stress is massive or prolonged.

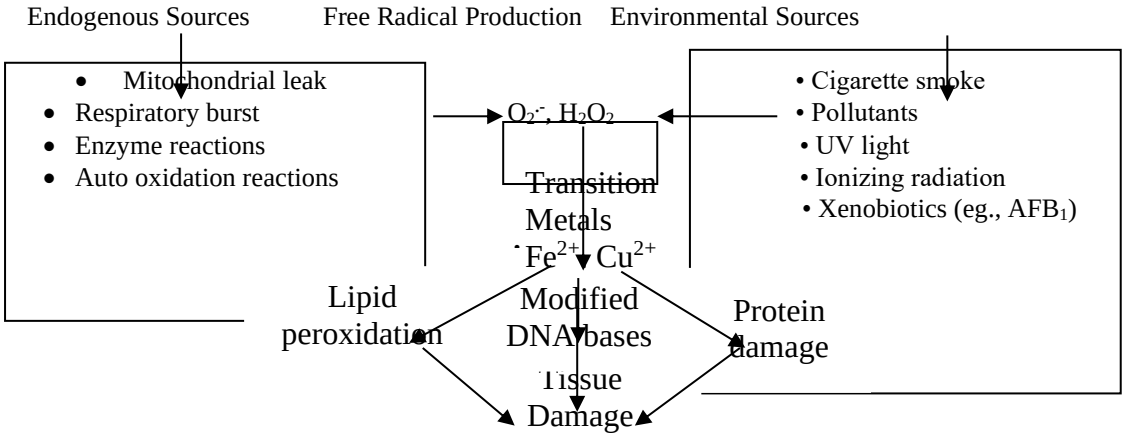


Fig. 1. Free Radical Production

Oxidative stress usually refers to the impairment of the function of cellular components (e.g. enzymes, nucleic acids, and proteins) by reactive oxygen species such as superoxide radicals ($O_2^{\cdot-}$), hydroxy free radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). These agents are capable to initiate or extend cell injury extracting hydrogen atom from polyunsaturated fatty acids and causes a degenerative process known as lipid peroxidation (10). Aflatoxin B_1 toxicity is believed to be mediated by the generation of toxic metabolites (11) that initiate lipid peroxidation (12).

Superoxide ($O_2^{\cdot-}$) is produced by the addition of a single electron to oxygen, and several mechanisms exist by which superoxide can be produced *in vivo* (13). The electron transport chain in the inner mitochondrial membrane performs the reduction of oxygen to water. During this process free radical intermediates are generated, which are generally tightly bound to the components of the transport chain. However, there is a constant leak of a few electrons into mitochondrial matrix and this results in the formation of superoxide (14).

$$O_2 + e^- \longrightarrow O_2^{\cdot-}$$

Hydrogen peroxide is not a free radical itself, (but is usually included under the general heading of reactive oxygen species (ROS)) but is a weak oxidizing agent that might directly damage proteins and enzymes containing reactive thiol groups. However, its most vital property is the ability to cross cell membranes freely, which $O_2^{\cdot-}$ generally cannot do (15). Therefore, H_2O_2 formed in one location might diffuse a considerable distance before decomposing to yield the highly reactive $\cdot OH$, which is likely to mediate most of the toxic effects ascribed to H_2O_2.

The $\cdot OH$ is possibly the final mediator of most free radical induced tissue damages (16). It is likely to react with almost every type of molecule found in living cells including sugars, amino acids, lipids and nucleotides. Although $\cdot OH$ formation occur in several ways, by far the most important mechanism *in vivo* is likely to be the transition metal catalyzed decomposition of $O_2^{\cdot-}$ and H_2O_2 (17) and these reactions involve transition metal ions [Fe^{3+}, Cu^{2+}] that are reduced by mechanisms involving sulfhydryl (-SH) groups on membrane proteins or by electron transport chain.

H_2O_2 react with iron II (or copper I) to generate the hydroxyl radical, a reaction first described by Fenton and iron-catalyzed Heber-Wiess reactions.

(i) Fenton Reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

(ii) Heber-Wiess Reaction

$$O_2^{\cdot-} + H_2O_2 \longrightarrow OH^{\cdot} + OH^-$$

Antioxidant defence against free radicals

Whenever a free radical interacts with another molecule, secondary radicals may be generated that can then react with other targets to produce yet more radical species (Pro oxidant state). Living cell evolved defences against free radicals and reduction of these peroxyl radicals by antioxidant molecules is crucial to the protection of cells against the development of pro oxidant state.

Enzymatic defenses against free radicals

Role of SOD

Any biological system generating superoxide will also produce H_2O_2 as a result of a spontaneous dismutation reaction. The superoxide dismutase catalyzes the dismutation of superoxide to hydrogen peroxides.

$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$

There are three forms of SOD in mammalian tissues, each with a specific subcellular location and different tissue distribution**.**

(1) **CuZn SOD** is found in the cytoplasm and organelles of virtually all mammalian cells (18).
(2) **Mn SOD** is found in the mitochondria of almost all cells. (19).
(3) **Extra cellular SOD** is a secretory enzyme that consists of copper and zinc that is distinct from the CuZn SOD (20).

Role of CAT

Catalase was the first antioxidant enzyme characterized and was found catalyzing the two stage conversion of H_2O_2 to water and oxygen (21).

Catalase – Fe (III) + H_2O_2 → Compound I

Compound I + H_2O_2 → Catalase –Fe (III) + $2H_2O + O_2$

Catalase is largely located within cells in peroxisomes, which also contain most of the enzymes capable of generating H_2O_2.

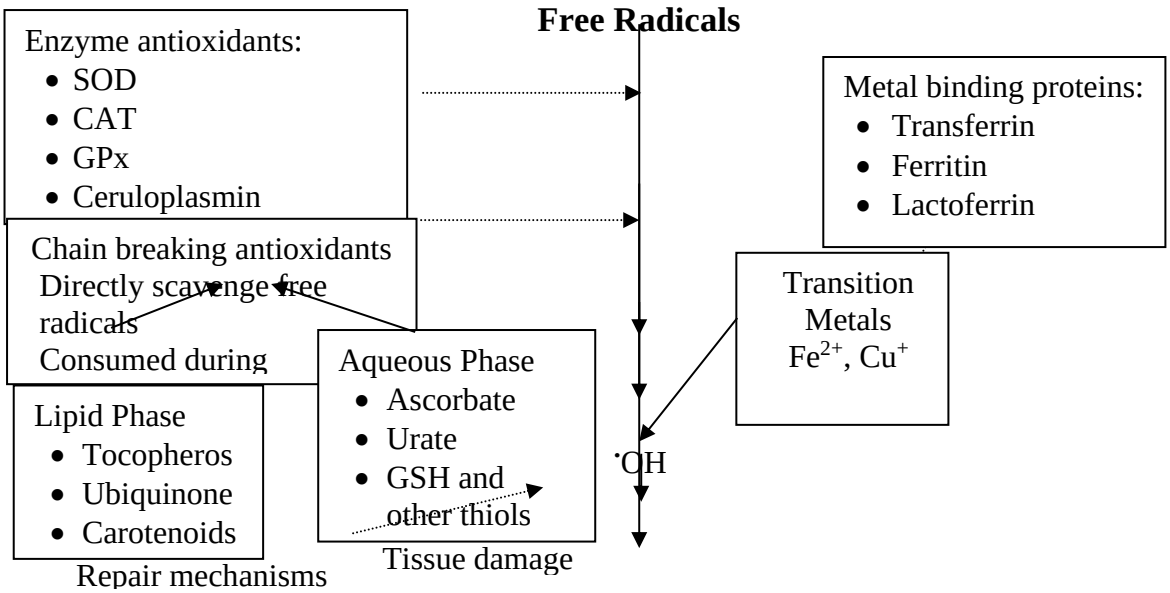


Fig. 2. Antioxidant defenses against free radical attack

Role of GPx

H_2O_2 generated in the mitochondria is detoxified by Glutathione peroxidase (GPx) and in the cytosol by both GPx and catalase. Glutathione peroxidase catalyzes the oxidation of glutathione at the expense of a hydroperoxide, which might be H_2O_2 or another species such as a lipid hydroperoxide (22).

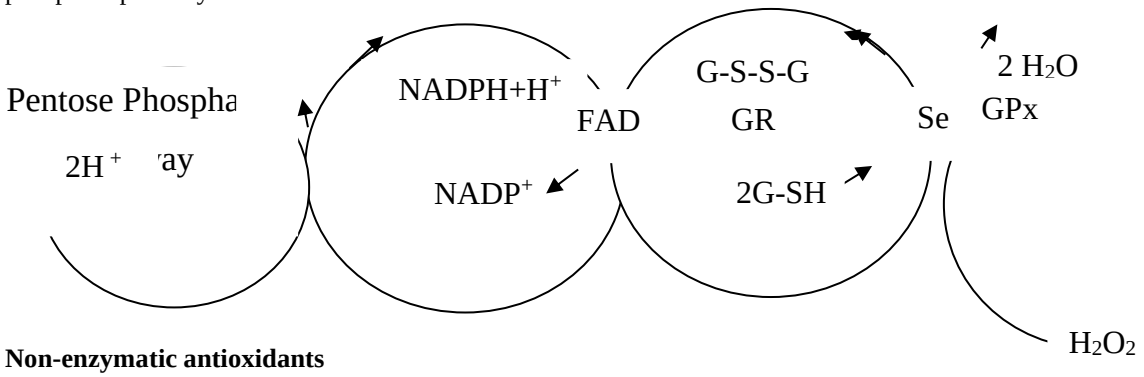
ROOH + 2 GSH → GSSG + H_2O + ROH (GPx)

Other peroxides, including lipid hydroperoxides, can also act as substrates for these enzymes, which might therefore play a role in repairing damage resulting from lipid peroxidation.

Within cells, the highest concentrations are found in liver although GPx is widely distributed in almost all tissues. The predominant subcellular distribution is in the cytosol and mitochondria, suggesting that GPx is the main scavenger of H_2O_2 in these subcellular compartments. The activity of enzyme is dependent on the constant availability of reduced glutathione (23).

Role of GR and Glucose-6-phosphate dehydrogenase

The oxidized glutathione (GSSG) produced by the action of GPx while scavenging H_2O_2 is further reduced to GSH with the action of NADPH. Generation of NADPH is the prime role of G-6-PDHase via., the pentose phosphate pathway.



Non-enzymatic antioxidants

The classic example of such a chain reaction is lipid peroxidation, and the reaction will continue to propagate until two radicals are neutralized by a chain breaking antioxidant (24). Chain breaking antioxidants are small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable byproducts (25). Such antioxidants can be conveniently divided into aqueous phase and lipid phase antioxidants.\

Vitamin E (α-Tocopherol)

The most important lipid phase antioxidant is probably vitamin E (26). They react more rapidly (than PUFA) with peroxyl radicals and trap them, hence act to break the chain reaction of lipid peroxidation (27). α - Tocopherol is the most abundant and potent antioxidant in humans. It quickly reacts with a peroxyl radical to form a relatively stable tocopheroxyl radical, with the excess charge associated with the extra electron being dispersed across the chromanol ring of the tocopherol. α - Tocopherol might be regenerated by reaction at the aqueous interface with ascorbate (28) or by reduced GSH or urate (29). Vitamin E, the major lipid soluble peroxyl radical scavenger and donating its labile hydrogen atom from phenolic hydroxyl groups propagates the lipid peroxyl and alkoxyl radical intermediates which can limit the rate of lipid peroxidation by terminating the chain reactions initiated in the membrane lipids (30). Das (1994) (31) reported that, vitamin E is the most significant antioxidant of animal cells and it can protect against carcinogenesis and tumour growth.

Vitamin C

The most important antioxidant of aqueous phase is vitamin C (ascorbate) (32). Ascorbate has been shown to scavenge superoxide, hydrogen peroxide, the hydroxyl radicals, and singlet oxygen. It reduces the chemical toxicity by decreasing the covalent binding of reactive intermediate, reducing quinones, eliminating free radical metabolites, inhibiting the formation of toxic nitrosamines and facilitating xenobiotic elimination by conjugation to glucuronides (33).

GSH and other thiols

The other major chain breaking antioxidant in plasma is the protein bound thiol groups. The sulfhydryl groups present in plasma proteins can function as chain breaking antioxidants by donating an electron to neutralize a free radical, with the resultant formation of a protein thiyl radical. Albumin is the predominant plasma protein and makes the major contribution to plasma sulfhydryl groups, although it also has several other antioxidant properties (34) by reacting with and neutralizing the peroxyl radicals (35).

Glutathione (GSH), a tripeptide consisting of γ- glutamyl -γ-cysteinylglycine, is the most abundant intracellular thiol compound, which is reported to be widely distributed in all mammalian tissues, (36, 37). GSH accounts for more than 90% of the total non-protein sulfur. The characteristic structural features of GSH are, the γ-Glu- linkage and -SH promotes its intracellular stability and are immediately associated with its functions.

GSH in reductive processes are essential for the synthesis and degradation of proteins, formation of deoxyribonucleotide precursors of DNA, regulation of enzymes, and protection of cells against reactive oxygen species and free radicals produced even in normal metabolism (Meister and Anderson, 1983; Sen and Hanninen, 1994).

Conclusion

AFB_1 epoxides are reported to conjugate readily with the cellular GSH with a concomitant inhibition of AFB_1-DNA adducts formation and protects tissue against AFB_1 induced carcinogenesis. Reduction in the level of GSH in tissues including liver makes the tissue to be more sensitive to the effect of the toxicant of interest.

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