

DNA Reactive Activities of Some Endogenous Metabolites and their Putative Role in the Induction of Cancer

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Abstract

One of the most intriguing scientific mysteries is the origin of cancer. DNA damage plays a potent role in mutagenesis, carcinogenesis and ageing. There has been growing realization in recent years that endogenous substrates or metabolites may be an important source of damage caused to cellular DNA. Further, evidence indicates that endogenous DNA damage occurs at a high frequency as compared to exogenous damage. Such findings have debunked the notion that the genetic material is pristine in the absence of exogenous carcinogens. An in-depth knowledge of the types of endogenous DNA damage is essential for understanding the influence of damage caused by endogenous substrates on the induction of cancer. Therefore, in this review, we have focused on several classes of endogenous metabolites and their putative role in mutagenesis and carcinogenesis from a structural, biological and analytical perspective. The mechanisms by which endogenous substrates may contribute to cancer pathogenesis have also been discussed with the objective that the implications from the findings summarised may pave way for a better understanding of the mechanisms underlying the induction of disease such as cancer. These results, complemented by other data, strongly support one of the theories of carcinogenesis according to which endogenous agents may have a major contribution in spontaneously induced cancer. It is obvious that if an approach could be developed leading to a diminution in endogenous DNA damage, the incidence of diseases such as cancer might be significantly reduced.

Keywords: DNA; Damage; Endogenous; Metabolites; Carcinogenesis; Mutagenesis

Introduction

Cancer is a disease that initiates with mutation of essential regulatory genes such as, oncogenes and tumor suppressor genes [1]. Successive accumulation of mutations in genes is considered to be basic to the process of carcinogenesis [2] (Figure 1). As to how the cancer causing mutations arise has been the question at the heart of the field ever since.

In the late 1960s, James and Elizabeth Miller proposed a hypothesis stating the basic principles of carcinogenesis [3,4]. They proposed that the first step in the multi stage phenomenon of tumorigenesis is the covalent binding of chemicals to cellular macromolecules, DNA, RNA and proteins. These chemicals or species are able to react with macromolecules owing to their electrophilicity. Since the publication of this hypothesis, researchers have been highly interested in elucidating the mechanisms underlying chemical carcinogenesis. Studies in this discipline have shown that carcinogenesis by several chemicals involves either a direct action of the chemical on cellular DNA or conversion of the parent chemical to a reactive form, which can then react with cellular DNA to produce permanent changes in the DNA [5].

DNA damage is known to play a potent role in mutagenesis and carcinogenesis [6] (Figure 1). Reaction of DNA bases with electrophilic agents, oxidising substrates, reactive metabolites or ultraviolet light produces covalently modified bases that are termed as DNA adducts [7-10]. Most of the research has focused on adducts derived from chemicals of exogenous origin such as hydrocarbons, aromatic amines, mycotoxins, etc. However, even in DNA of normal cells, several categories of endogenous adducts have been detected. Such adducts include oxidized bases, alkylated bases, exocyclic base adducts, and a range of unidentified adducts referred to as I-compounds [11]. Identification of these adducts by various techniques has led to an understanding of why and how they can initiate cancer [12,13].

The etiology of most human cancers remains unknown but it is presumed that the major carcinogenic risk to humans is posed by endogenous carcinogens. DNA mutation is an essential step in carcinogenesis and increased levels of DNA lesions have been detected in various tumors, implying the role of such damage in the etiology of cancer [14]. It is known that oxidatively modified DNA is abundant in many human tissues, especially in tumors [15,16].

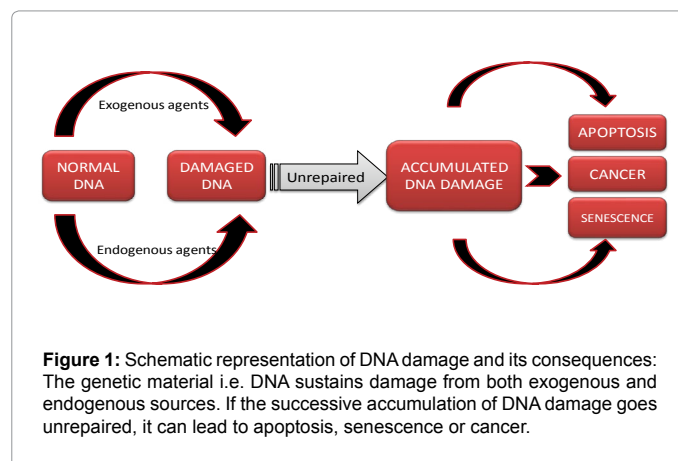


Figure 1: Schematic representation of DNA damage and its consequences: The genetic material i.e. DNA sustains damage from both exogenous and endogenous sources. If the successive accumulation of DNA damage goes unrepaired, it can lead to apoptosis, senescence or cancer.

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Received June 30, 2015; Accepted August 21, 2015; Published August 25, 2015

Citation: Rehmani N, Hadi SM (2015) DNA Reactive Activities of Some Endogenous Metabolites and their Putative Role in the Induction of Cancer. J Cancer Sci Ther 7: 283-291. doi:10.4172/1948-5956.1000364

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Historically, research into the role of DNA damaging agents has focused primarily on toxic chemicals of exogenous origin, attention is now being shifted to by-products of endogenous origin [17]. Evidence indicates that endogenous DNA damage occurs at a high frequency as compared to exogenous damage and the types of damage caused as a result of endogenous cellular processes are identical or similar to those produced by various exogenous agents [18]. This emphasis on the role of endogenous metabolites as DNA damaging agents is due to the technological developments that are able to detect adducts of endogenous origin in cellular DNA [19-23]. While studying the role of exogenous carcinogens in animal models, analogous DNA adducts were also found in control groups [24]. Research studies like this triggered the search for the physiological substrates that produced these DNA adducts and other pathways that lead to endogenous genotoxicity.

There is increasing evidence that considerable DNA damage is caused by adducts produced from endogenous substrates that arise as a result of normal or aberrant metabolism. For example, malondialdehyde which is produced as a result of lipid peroxidation and eicosanoid metabolism has been shown to react with cellular DNA to form a propanodeoxy-guanosine adduct [25,26]. Further, there has been evidence that this adduct exists at significant levels in the DNA of rats and humans and is also an efficient mutagen in *E.coli* [25,26]. Thus, there is strong implication that adducts formed by endogenous substrates may contribute to the etiology of genetic diseases and therein the baseline of human cancer.

Based on the multitude of evidences on the role of endogenous metabolites as DNA damaging agents, studies in our laboratory have also shown that several classes of endogenous metabolites such as L-DOPA [27], Uric acid [28], Serotonin [29], Melanin [30], and Bilirubin [31], etc. are capable of causing DNA breakage. We have also proposed a probable mechanism for the genotoxicity induced by these endogenous substrates (Figure 2). Our studies suggest that these metabolites are capable of binding to DNA as well as Cu (II). A ternary complex of DNA-Cu (II)-endogenous metabolite may thus be formed owing to such binding affinities. These metabolites have also been shown to generate harmful reactive oxygen species either alone or in the presence of transition metal ions such as Cu (II) which ultimately cause cellular DNA breakage. We have earlier proposed that several antioxidants, both of plant and animal origin such as uric acid [28], tannic acid [32,33] and flavonoids [34] are capable of acting as pro-

oxidants under appropriate conditions or microenvironment. Our studies provide strengthening evidence for the role of the endogenous chemicals as genotoxins in spontaneously induced cancer.

In this review, we have discussed several classes of endogenous metabolites in relation to their putative DNA damaging potentials. The findings will hopefully help frame questions about the risks posed by endogenous metabolites and encourage further research to answer some of these questions. Further, it is hoped that this study will provide insight into the contribution of endogenous DNA damage which leads to mutagenesis, wherein lies the baseline of human cancer.

Pro-oxidant and antioxidant properties of uric acid

Uric acid (2, 6, 8-trioxopurine) (Figure 3) is formed in mammals as an end product of purine metabolism [28]. It is derived exclusively from the oxidation of xanthine and hypoxanthine by the enzyme Xanthine Oxido Reductase [35]. In the human plasma, uric acid is present in saturating concentrations (up to 0.6 mM) [36], making it one of the major antioxidants in humans. Numerous studies have shown that uric acid is able to scavenge singlet oxygen [37,38] as well as hydroxyl radicals [39].

Antioxidant potential of uric acid

Over the past 60 million years, a marked increase in life-span has occurred in human evolution [40]. It seems evident that the evolution of protective mechanisms against oxygen radicals may have been a major factor in increasing the life span of humans and decreasing the rates of age related cancers [40-46]. In 1982, Ames et al proposed that uric acid provides a primary defence against human cancer and it may be partly responsible for relatively long life span of humans based upon its high serum concentration, its capacity to scavenge singlet oxygen and to inhibit lipid peroxidation in humans [47]. Since the publication of this hypothesis, numerous reports documenting the role of uric acid as an antioxidant were generated in the ensuing years [48].

Uric acid and disease risk

There has been increasing concern that hyperuricemia may have a significant role in various disorders such as hypertension [49], acute renal injury [50], cardiovascular disease [51-53] and MetS-metabolic syndrome [54-56]. Owing to its low solubility, elevated uric acid in blood gets deposited in tissues leading to some disease conditions like

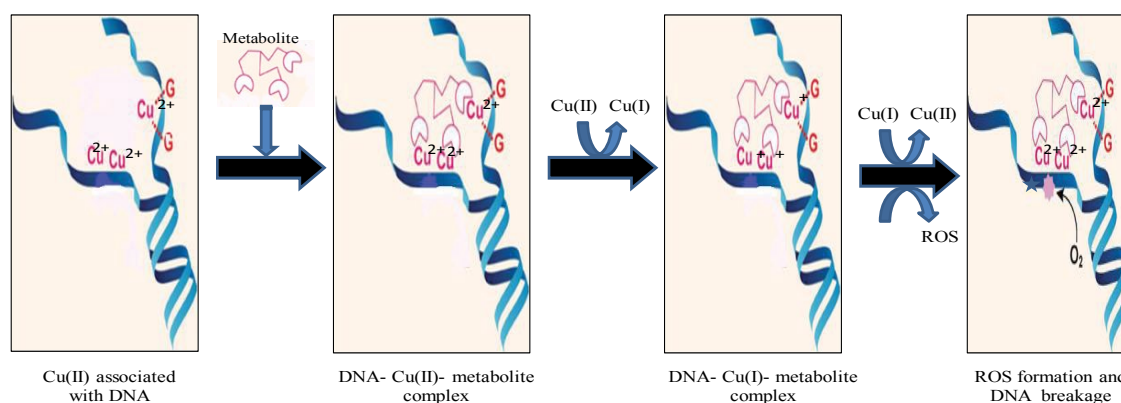


Figure 2: The schematic model proposed for the formation of a ternary complex: the redox cycling of endogenous metabolite, DNA and copper ion complex leads to generation of reactive oxygen species (ROS) which inflicts breakage in DNA.

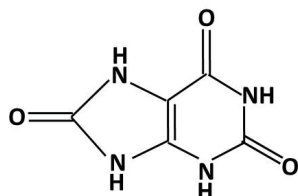


Figure 3: The chemical structure of uric acid (2, 6, 8- trioxypurine).

gout or rheumatoid arthritis [28]. Although uric acid is known as a potent antioxidant, recent studies have demonstrated that elevated serum uric acid is independently and significantly associated with excess cancer risk and mortality [57]. There is a relevant correlation of hyperuricemia with augmented risk of certain cancers like colorectal, breast and prostate and others [58-62]. It has been suggested that its pro-inflammatory properties may have an important contribution in the pathogenesis of cancer [57]. Furthermore, statistical data suggests that elevated serum uric acid is strongly associated with increased premature cancer death in both men and women [63-65]. In a study involving patients with terminally end stage cancer, high levels of serum acid were predictive of reduced survival time of the patients [66]. Evidence indicates that there is a dose dependence and time varying link of serum uric acid with cancer mortality [67]. Based on extensive data, it has been proposed that excess uric acid (intracellular and extracellular) contributes to tumorigenesis. According to this model, elevated serum uric acid when present extracellularly, stimulates tumor cell proliferation and migration and it induces inflammatory stress causing transformation, when it enters the cell [57]. Such studies are implicative of a more intricate role of uric acid in cancer biology than that of a general antioxidant.

Pro-oxidant effects of uric acid

It is known that uric acid is able to bind Cu (II) [68]. Our studies on uric acid have demonstrated that it can cause breakage of calf thymus DNA and supercoiled plasmid DNA in the presence of Cu (II) and molecular oxygen. We have shown that uric acid generates hydroxyl radicals in the presence of Cu (II) and it also causes the reduction of Cu (II) to Cu (I) which is an essential intermediate in the DNA cleavage reaction. The involvement of oxygen species in the reaction was confirmed by the inhibition of DNA breakage in the presence of oxygen radical scavengers [28].

Based on the abovementioned studies, we have proposed the formation of a ternary complex by the uric acid, DNA and Cu (II) system which could damage DNA. There is also a possibility of formation of urate anion by the reaction of uric acid with oxygen radicals. The urate anion has been shown to inactivate enzyme systems and thus may be able to damage DNA [69]. Our results do not detract from the established antioxidant role of uric acid in extracellular fluids. Instead, our studies support the idea that several antioxidants (in this case, uric acid), are able to act as pro-oxidants under appropriate conditions.

DNA reactive activities of bilirubin and its metabolic precursor biliverdin

Biliverdin (Figure 4A) and bilirubin (Figure 4B) are heme degradation products. Heme is degraded to biliverdin by the action of heme oxygenase. Biliverdin is further reduced to bilirubin mediated by biliverdin reductase [70]. These bile pigments have been attributed

with harmful as well as beneficial properties. Both bilirubin and biliverdin have been reported to play antioxidant roles in the body based on their radical scavenging properties [71]. For example, it has been shown that these compounds can prevent superoxide production [72], quench singlet oxygen [73], inhibit lipid peroxidation [74] and scavenge peroxynitrite [75,76].

Biliverdin is considered as a soluble and non-toxic compound [77]. Although, some studies have indicated that it can have toxic roles. It has been found that biliverdin concentrations are significantly increased in hepatic necrosis [78]. Evidence indicates that biliverdin can also cause overexpression of some oncogenes in the liver [79]. It has also been associated with Bronze Baby syndrome [80].

Normal plasma concentrations of bilirubin range from 5 to 17 μM [81] almost all of which is bound to albumin [82] or exists as a conjugate with glucuronic acid [83]. Albumin bound bilirubin is considered one of the naturally occurring antioxidants of human extracellular fluids [68]. It was the pioneering study by [84] which launched the idea that endogenous bilirubin is one of the most potent antioxidants in the serum. Further studies have demonstrated that bilirubin reduces oxidative stress in neurons [85] and cardiomyocytes [86].

It has also been found that bilirubin functions as a physiological neuroprotectant [85]. As reported by Hayashi et al., bilirubin also exerts anti-inflammatory effects [87]. Contrary to these findings, several studies have shown that bilirubin is toxic to cells in vitro as well as in vivo [88,89]. Increased levels of bilirubin in plasma are known to be related with disease conditions such as jaundice and hyperbilirubinemia [83]. Further, it has also been found that elevated concentrations of bilirubin (greater than 300 μM) may contribute to the development of neurological dysfunctions [90] as well as impairment of cellular functions in brain [91]. Inhibition of various membrane bound enzymes by bilirubin has also been reported [92].

Evidence for pro-oxidant activity

In vitro studies in our laboratory have aimed at elucidating the

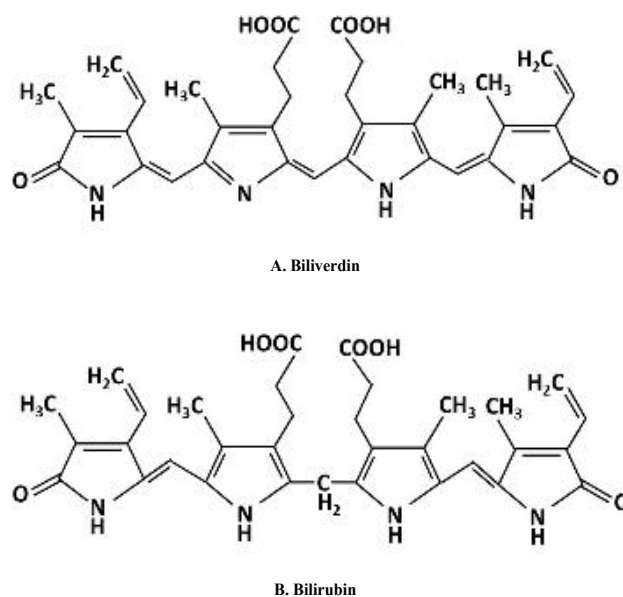


Figure 4: The chemical structures of A) biliverdin and its metabolic derivative B) bilirubin.

mechanism of DNA breakage reaction by these bile pigments. Our results have indicated that bilirubin is able to cause strand scission in DNA in the presence of Cu (II) via generation of reactive oxygen species [31]. Subsequently we compared the antioxidant and prooxidant activities of biliverdin and its metabolic derivative bilirubin [71]. Our findings imply that bilirubin, as compared to its metabolic precursor biliverdin is more efficient as an antioxidant and also as a prooxidant. Although there is enough evidence in the literature implicative of the toxic effects of these metabolites, the present literature is not sufficient to explain whether the elevated levels of these bile pigments are the result or cause of the toxicity.

Role of estrogens and their metabolites as genotoxic agents

Naturally occurring estrogens (Estrone E1, Estradiol E2, Estriol E3) are a group of steroidal compounds (Figure 5). Although the physiological action of estrogens occurs at hormone responsive organs (testis, uterus, pituitary), they also exert some action at other organs (kidney, liver etc.) as well [93]. Numerous *in vitro* and *in vivo* studies have demonstrated that estrogens possess antioxidant properties [94]. It has been established that all estrogens at their micromolar concentrations exert antioxidant action [95-97].

Earlier research categorised estrogens as non genotoxic due to the inability of these metabolites to induce gene mutations in classical bacterial and mammalian mutation assays [98-100]. Nevertheless, with the advancements in sensitive methods to detect adducts and a thorough understanding of the estrogen metabolism, there has been strengthening evidence in support of estrogen mediated genotoxicity [101-103]. Pharmacological levels of estrogens have been shown to produce adverse effects such as embryotoxicity, teratogenicity and carcinogenicity [104,105]. Studies on animal models have documented that estrogens can act as tumor initiators [106,107]. Further, elevated concentrations of estrogens have also been found to be associated with cancer in humans.

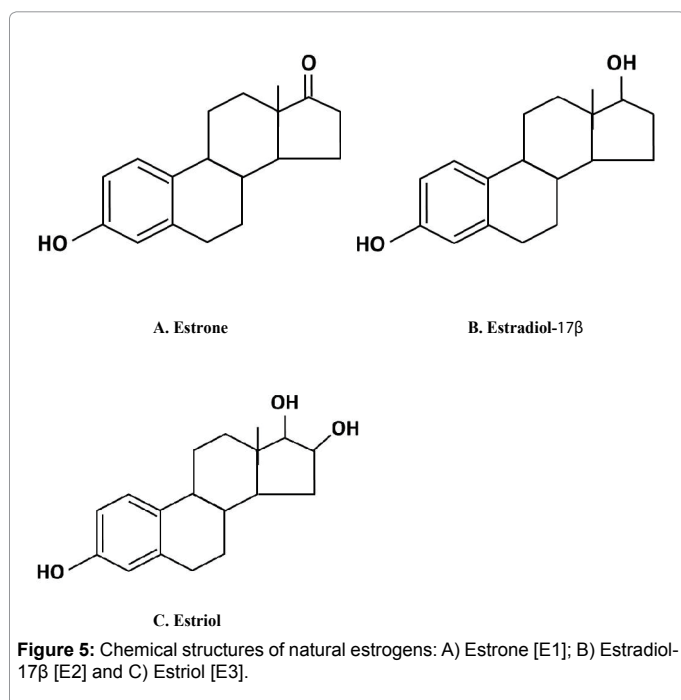


Figure 5: Chemical structures of natural estrogens: A) Estrone [E1]; B) Estradiol-17β [E2] and C) Estriol [E3].

In 1960s and 1970s, the initial evidence for the DNA binding activity of estrogen metabolites was obtained [93]. In the ensuing years, more studies started shedding light on the diverse types of genetic insults caused by estrogens and their metabolites. It has been reported that the estrogens, estrone and estradiol are able to react with DNA as well as proteins [108,109]. Although the estrogen hormones are not able to link covalently with DNA *per se*, but their reactive metabolites are known to form covalent bonds with nucleotide bases [93]. Catecholestrogens, the major metabolites of the estrogens are known to oxidise to yield quinones which may form adducts by reacting with DNA. A hypothesis holds that these adducts may generate mutations that might initiate many human cancers [110]. Stack et al. [111] and Cavalieri et al. [112] have shown that various metabolites of estrogens, specifically estrone 3, 4-quinone are able to form unstable adducts by reacting with DNA bases. In an *in vivo* study involving hamsters, it was found that exposure to estrogens for several months resulted in the enhancement of DNA adducts in kidney of the hamsters [113-115]. In another experiment, co-administration of estrogen with an anti-estrogen failed to affect the increase in adduct levels caused by estrogens [116].

Active oxygen radicals are known to be produced by the redox cycling of catecholesterogen metabolites [93]. Microsomal enzymes such as P450 are capable of catalysing the redox cycling of estrogen metabolites and thus generate oxygen radicals [117-119]. In a recent study, Seacat et al. [120] have shown that metal ions such as Cu (II) can also catalyse the oxidation of catecholestrogens generating hydroxyl radicals. The free radicals produced by redox cycling of estrogens enzymatically or non-enzymatically can induce DNA damage by different mechanisms [93]. For example, Nutter et al [121,122] have shown that the free radicals produced by the oxidation of estrogen quinones in cells are capable of inducing single strand breaks in DNA. Further, the reactive species produced by catecholestrogens have also been implicated in causing structural aberrations in the chromosomes [93].

Although, the parent estrogen hormones do not possess reducing properties, estrogen metabolites such as catecholestrogens are capable of reducing metal ions such as Cu^{2+} to Cu^{+} or Fe^{3+} to Fe^{2+} [94]. The reducing capability of catecholestrogens may be attributed to their catechol structure which is absent in the parent hormones. It can be presumed that the mechanism of the pro-oxidant effect of catecholestrogens is based upon their capability of reducing transition metal ions such as Cu (II) to Cu (I) ions which has been shown to initiate lipid peroxidation via generation of free radicals [123]. Evidence has also indicated the pro-oxidant effect of estrogens metabolites *in vivo* [124-126].

Altogether, these studies emphasize the genotoxic effects of estrogen which have received little attention in the past. These findings are implicative of the possible role of estrogens in the complex process of hormone-induced carcinogenesis.

Contribution of neurotransmitters in carcinogenesis

Serotonin (5-hydroxytryptamine) is biochemically derived from the amino acid, tryptophan (Figure 6A). It is an important neurotransmitter in brain and spinal cord and affects several behaviours such as hunger, aggressiveness, sleep, mood and consciousness [127]. Studies in our laboratory have demonstrated that serotonin can cause strand breakage in DNA in the presence of Cu (II) via an oxidative mechanism [29]. We have shown that serotonin catalyses the reduction of Cu (II) to Cu (I) and this reaction is accompanied with hydroxyl radical formation. In order to elucidate the chemical basis of the DNA

breakage by serotonin, we also compared the efficiency of DNA cleavage of serotonin with its structurally related metabolites i.e. tryptophan and melatonin [127]. It has been reported that copper ions can bind to serotonin as well as its metabolite melatonin [128] (Figure 6B). Our studies indicated that only serotonin-Cu (II) complex is efficient in degrading DNA as compared to melatonin-Cu (II) or tryptophan-Cu (II). Based on our results, we have proposed that the phenolic group present in serotonin is important in causing DNA strand cleavage in the presence of Cu(II) and that this interaction involves the formation of quinone methide [127].

L-DOPA (L-3,4 dihydroxyphenylalanine) (Figure 6C) is formed from the hydroxylation of the amino acid, L-tyrosine mediated by the enzyme tyrosine hydroxylase. It serves as a metabolic precursor in several metabolic reactions such as the synthesis of melanin, dopamine, adrenaline and nonadrenaline. The nervous system is extensively rich in L-DOPA and its metabolites (dopamine, 3-o-methyl dopa) [129]. L-DOPA is prescribed as a drug to Parkinson's patients for replenishing the loss of dopamine in human brain. In 1994, Halliwell and co-workers had proposed that L-DOPA and its metabolites can cause extensive DNA damage in the nervous system. In the ensuing years, numerous *in vitro* and *in vivo* studies were reported documenting the toxicity of L-DOPA and its metabolites.

Dopamine (Figure 6D) is formed from L-DOPA by the action of dopa decarboxylase. It is a chemical neurotransmitter in the central nervous system and accounts for 90% of the total catecholamines [27]. The dopaminergic system is involved in regulating various physiological processes such as emotion, cognition, neuromodulation, etc. [130-132]. Evidence has indicated that dopamine is capable of interacting with DNA [133]. It has been proposed that the availability

of redox active catecholamines such as dopamine in the presence of copper ions may lead to cytotoxicity in the brain [129]. Further, the oxidative metabolism of dopamine and other catecholamines has been implicated in the generation of free radicals and quinines [134-136]. Numerous studies have documented the toxicity of dopamine and its metabolites in relevance to Parkinson's disease [137-140]. Thus, it is a matter of concern that the current drug for Parkinson's disease i.e. L-DOPA may increase the risk of such oxidative damage [141]. It has also been reported that dopamine can induce apoptosis in cells and thus it is implicated to possess antitumor properties [142,143].

Dopamine combines with acetaldehyde, to yield 1-methyl-6,7-dihydroxy-1,2,3,4 tetra hydroisoquinoline (Salsolinol) [144,145] (Figure 6E). This metabolite has been identified in rats and human brain tissues [146,147]. It has been speculated that salsolinol plays an important role as a neuromodulator of catecholaminergic neurotransmission [148]. It has been found that the neuromodulator function of salsolinol bears resemblance to L-DOPA remarkably [149-151]. Although, convincing evidence indicates that salsolinol and its metabolites possess neurotoxic properties [148]. (For example, salsolinol and its metabolites have been implicated to be involved in the pathogenesis of Parkinson's disease [152]. It has been reported that salsolinol can induce accelerated apoptotic cell death in the presence of copper in neuroblastoma cells [153]. Evidence indicates that salsolinol can elicit apoptosis, possibly via generation of reactive oxygen species [154]. Interestingly, it is has been noted that salsolinol may either decrease or increase the generation of hydroxyl radicals [148]. Therefore, it has been postulated that salsolinol represents a double-faced molecule that can act either as a neuroprotector or a neurotoxin. Hypothesis holds that the disbalance in the proportion of neuroprotectivity and neurotoxicity of salsolinol leads to diseases such as Parkinson's disease [155].

Melanin is synthesised from L-DOPA via the polymerisation of DOPA chrome [156]. This metabolite is known to protect the skin from solar carcinogenesis [30]. Although, it has also been found that melanin can generate free radicals and also cause oxidative DNA damage [30]. Evidence has indicated that melanin on UV irradiation produces base modification and strand breaks in whole cells [157,158]. Melanin has also been shown to bind to several metal ions such as iron, copper and zinc [159]. Studies in our lab have shown that melanin is able to damage DNA in the presence of copper ions mediated by ROS [30].

Conclusion

The high prevalence of sporadic cancer evidenced in our population cannot be justified on the basis of mutagenic capability and concentrations of the exogenous carcinogens present in our environment [160]. Thus, it is evident that mutations due to DNA damage by endogenous sources must play a significant role in most cases of cancer, in addition to exposure to exogenous carcinogens. In the past two decades, the discovery of considerable DNA damage occurring from endogenous sources [161] has provided the major impetus to the field of carcinogenesis research. Subsequently, research focused on the damage incurred by endogenous agents on DNA and the consequences thereof. Several investigations have documented the impact of endogenous background DNA damage on human cancer [6,161,162].

The picture emerging from this review reveals that the genome is vulnerable to hazardous cellular environment, particularly to the incessant flux of reactive products generated by normal or aberrant metabolism. These metabolites can induce damage to DNA by a variety of mechanisms, as discussed in this review. While such substances

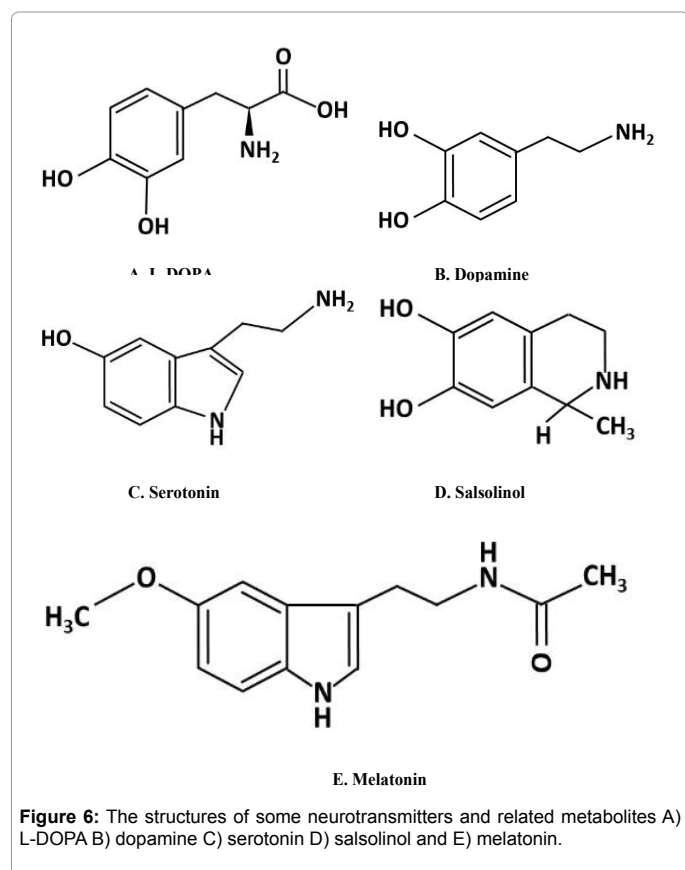


Figure 6: The structures of some neurotransmitters and related metabolites A) L-DOPA B) dopamine C) serotonin D) salsolinol and E) melatonin.

may not possess the potency of several exogenous carcinogens, it can be presumed that lifelong exposure to endogenous toxicants results in accretion of cell damage that leads to many disorders including cancer [163]. It is conceivable that the cumulative effect of endogenous metabolites may be of physiological significance and could contribute to etiology of genetic diseases. Taken together, these findings imply a major contribution of DNA damage in mutagenesis and carcinogenesis.

We are left to grapple with several questions pertaining to the risk contributed by endogenous agents. Is the effect of DNA adduction arising from endogenous agents, additive, synergistic or antagonistic to that caused by exogenous carcinogens? What are the interactions of endogenous metabolites with xenobiotic agents? Also, what are the key steps in the carcinogenesis process that are triggered by endogenous metabolites? Distinguishing between exogenous and endogenous DNA damage is an intricate matter as some exogenous agents also occur endogenously and generate same DNA adducts as endogenous metabolites. While evaluating the contribution of endogenous metabolites in inducing DNA damage, a point worth being taken into consideration is that some of these substances are produced from more than one source. Unfortunately, due to technical and theoretical restrictions in current understandings of cancer origin, answers to such questions are presently beyond our grasp. These matters need to be addressed before the biological significance of endogenous metabolites may be fully appreciated.

It is expected that developments in analytical methodology will continue to progress at a swift pace, enabling sensitive ways to detect and quantitate endogenous DNA damage. Further, we need to accumulate more information on the biological consequences of DNA damage that arises due to endogenous processes. Such findings are crucial to the development of novel chemopreventive strategies. It is obvious that if an approach could be developed leading to a diminution in endogenous DNA damage, the incidence of cancer may be significantly reduced.

Acknowledgements

We want to admittedly mention that although we tried to provide a comprehensive overview on the subject, we might have inadvertently missed few good studies and relevant observations related to the field of study.

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of this manuscript.

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