
Review

Antimutagenesis Studies: Where Have They Been and Where Are They Heading?

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An anti-mutagen is any substance that reduces the rate of spontaneous mutations or counteracts or reverses the action of a mutagen, or any technique that protects cells against the effects of mutagens. Studies as early as the 1940s reported on substances that delayed detection of radiation-induced mutations, or reduced the appearance of mutations induced by chemicals such as acridine orange. However, a far more sophisticated range of anti-mutagens is now being identified. Mutagen scavengers act through absorption onto a larger molecule that is readily excreted. Good examples are provided by dietary fibre sources, such as wheat bran, or the planar molecule, chlorophyll and its stabilised derivative, chlorophyllin. Mutagens may be actively extruded from human cells through the action of one or more of a series of ATP-binding cassette (ABC) drug transporter proteins, including the multidrug resistance proteins (P-glycoproteins), multidrug resistance-associated proteins (MRP1–7) and the breast cancer resistance protein (BCRP). These proteins can affect the absorption, distribution and excretion of mutagens and carcinogens, as well as of their metabolites and conjugates. Even if the undesirable compound enters the cells, there are several mechanisms by which it may be prevented from interaction with DNA. Detoxification mechanisms are of increasing interest, especially those where transcription is regulated through the antioxidant response element (ARE), whose own transcription factor, Nrf2, is repressed under basal conditions. While much of the early literature on mutagenesis and carcinogenesis implicated exogenous chemicals, it is increasingly realised that unrepaired oxidative DNA lesions are important mutational precursors, and anti-oxidants represent an important class of anti-mutagens. It is also recognised that deficiency of certain micronutrients may lead to cell mutation, and that restoring nutrient balance is an important mechanism of anti-mutagenesis. An increasing number of studies focus on DNA repair and stress responses as novel mechanisms of anti-mutagenesis.

Key words: anti-mutagen, anti-carcinogen, chemoprevention, anti-oxidant, micronutrient

Introduction

A mutagen is “a physical or chemical agent that changes the genetic material, usually DNA of an organism and thus increases the frequency of mutations above the natural background level” (1). Mutations can be primarily divided into chromosomal or genome mutations, that affect a part or the whole of a chromosome, or gene mutations that affect the bases in the DNA of a single gene.

In the same way that a wide range of chemical and physical agents act as mutagens, there are a range of substances that can counter these effects. An anti-mutagen is considered to be “any substance that reduces the rate of spontaneous mutations or counteracts or reverses the action of a mutagen, or any technique that protects cells against the effects of mutagens” (1). This implies that specific types of mutagen are likely to be counteracted by specific anti-mutagens.

This early definition implied that mutagenic agents, of various types, lead to mutations. However, it is increasingly clear that nutrient deficiencies may act on chromosomes in the same way as mutagenic agents, such as ionising radiation (2,3). In this context, the addition of a micronutrient to restore the optimal nutrient balance may have strong anti-mutagenic effects. It is, however, always important to recognise that nutrients have optimal levels in a cell, and too much may be as damaging as too little (4).

Early Studies on Anti-mutagens

Anti-mutagens have been described since the 1940s, although not necessarily described as such. For example, studies by Newcombe (5), sometimes in association with Scott (6) led to the identification of substances that delayed the detection of radiation-induced mutations. Novick and Szilard originally reported what was then a new phenomenon, that certain nucleosides can act as
anti-mutagens in preventing the mutagenic action of various purine derivatives on bacteria (7). By 1956, Novick had been able to produce a general review on mutagens and anti-mutagens (8). Much of this early work focussed on chemicals that delayed cell growth, which was seen as an important mechanism of anti-mutagenesis.

By the 1960s, some newer anti-mutagens were being discovered, and the complexity of the field was becoming apparent. For example, Magni and coworkers described 5-aminoacridine (now known as 9-aminoacridine) as an anti-mutagen during mitosis but a mutagen during meiosis (9). Even that was an oversimplification, and we might more commonly consider this chemical as a frameshift mutagen, even as a classic frameshift mutagen rather more than an anti-mutagen (10,11). Some of the earlier studies also recognised that nutrient deficiencies could damage DNA, and that supplementation with nutrients such as methionine could provide an effective anti-mutagenesis regime (12). It is also of interest that some of the work on anti-mutagenesis in the 1960s had already recognised the significance of variant DNA polymerases, that may slow cell growth and division, and allow more accurate DNA repair (13).

The field has expanded enormously since the 1940s through to 1960s. A wide range of anti-mutagenesis mechanisms have been identified, often through work on cancer prevention (14–18). There is excellent evidence for the basic cellular mechanism of mutation comprising DNA damage, and ineffective repair or failure of cell loss by apoptosis leading to permanent DNA damage in some form (19,20). One of the key factors in the accuracy or otherwise of DNA damage leading to mutation is the time is necessary for accurate repair. Chemicals that slow the cell cycle may appear anti-mutagenic, but may only delay mutation. However, they may also allow sufficient time for error-free repair to occur, in which case they can be considered as true anti-mutagens (1,21–23). A wide range of other mechanisms are, however, becoming apparent, particularly involving stress mechanisms and a range of signalling pathways (24,25) (Fig. 1).

Examples of Anti-mutagens and Their Sources

There are large number of descriptive examples of anti-mutagen now in the literature. Some of these involve crude extracts, without chemical identification of the active constituent. For example, phenolic extracts from Andean purple corn (Zea mays L.) (26), various fruits and vegetables (27), a lichen extract (28), various extracts from Thai vegetables (29), from the thorns of Gleditsia sinensis (30), or water extracts from the mushroom, Agaricus blazei Murrill (31). More advanced methods of obtaining extracts, such as supercritical CO₂ extracts of Terminalia catappa leaves (32), are also yielding potentially novel anti-mutagens.

**Fig. 1.** An overview of some of the mechanisms of antimutagenesis and their role in the progression of a cell towards cancer. Antimutagens may directly interact with mutagens or mutagen precursors, to inactivate them or to prevent cellular uptake. Other types of antimutagen act at the cellular level by stimulating the action of one or more of a series of ATP-binding cassette (ABC) drug transporter proteins, which actively extrude potentially DNA-reactive molecules. Detoxification mechanisms include those where transcription is regulated through the antioxidant response element (ARE). As the cell accumulates mutations, it moves into an increasing condition of genomic instability, which may be enhanced by micronutrient deficiencies.
A number of other studies focus on specific classes of chemical constituents such as hydroxycinnamic acids from plant cell walls (33,34), citrus flavonoids (35), flavones from the roots of *Scutelleria baicalensis georgi* (36) or chamomile essential oil (37). Chemical isolation of the active compound is increasingly the method of choice. For example, 4-(methylthio)-3-butenyl isothiocyanate has been found to be the primary anti-mutagen in daikon (*Raphanus sativus*; Japanese white radish) (38), curcumin and its natural analogues (39–41), vanillins (25), 1,4-dihydroisonicotinic acid (42) or paraaminobenzoic acid (23). While many of these have been extracted from natural sources, chemical synthesis of anti-mutagens is also a common practice, especially as a potential means of producing chemopreventive agents (40,43–45).

**Examples of Anti-mutagen Protection against Different Classes of Chemicals**

**Protection against free radical generating agents, such as hydrogen peroxide:** Free radicals occur at a low but measurable level in any living organism, as a result of metabolism (46,47). They also occur at an increased rate as an individual ages, in certain human disease states and at an increased rate following exercise. However, there are a number of ways of protecting against free radicals. Exogenous dietary antioxidants interact with endogenous antioxidants to form a cooperative network of cellular antioxidants, including free radical scavengers. A range of cell types also contain enzymatic cellular defence mechanisms to eliminate free radicals, including inhibitors of pro-oxidant enzymes, and inducers of endogenous defences (14,48,49). All of these classes of agents may be considered anti-mutagens.

Good examples of anti-mutagens that may act as antioxidants are plant polyphenols (50), although it should be noted that these have other actions. Notable among the literature on antioxidants are studies on various teas, especially green tea (51,52). For example, polyphenols in green tea include catechins (flavan-3-ols) (53,54), while in black tea, theaflavin formation may be important (54,55). Polyphenols from green and black tea are effective antioxidants in model test systems, while tea polyphenols may also chelate pro-oxidant metals (56). However, the evidence for a link between tea consumption and health from epidemiological and clinical studies is still mixed (57). Tea polyphenols can be detected in the body after tea consumption (up to 2%, peaking after 1–2 h) (57). The dihydroxy or trihydroxy structure on the flavonoid B-ring is a key for effective free radical scavenging (58).

Other food sources also show antioxidant properties. For example, we considered a range of different red-purple coloured vegetables in the New Zealand diet, to show very potent antioxidant properties, occurring through various mechanisms (49,59). Some of these pigments are contained in the vacuole of a cell, where they will be readily available when the food plate is masticated. Others, however, are attached to plant cell walls, such as in the skin of a purple sweet potato or kumara (60). These may prove more effective antioxidants in protecting human health, since they may be released specifically in the colon, following digestion and fermentation by colonic bacteria.

**Protection against DNA-intercalating mutagens, such as 9-aminoacridine:** Intercalating agents were commonly used as surface antibacterial agents, although they now have more limited laboratory use. The “classic” laboratory antibacterial agent is also the archetypal frameshift mutagen, 9-aminoacridine (10,11). The mechanism of mutagenesis is likely to be far more direct than for DNA-reactive chemicals, or agents that are metabolised into a DNA reactive form (10,11). It seems likely that agents which stabilise the local structure of DNA, such as DNA minor groove binding drugs, will act as antimutagens (61). Other mechanisms which operate against DNA-reactive chemicals (see next section) may also be appropriate.

**Protection against DNA-reactive mutagens, such as heterocyclic amines:** Dietary sources of heterocyclic amines include well cooked red meats (grilled, shallow fried, barbequed), well cooked fish, well cooked chicken, and also other high protein foods after high cooking temperatures for long times (62,63). As these authors point out, such exposures may be unavoidable. Thus, it is important to consider how to protect against dietary mutagens such as heterocyclic amines. The most obvious method is to avoid their production, through modifying cooking methods. It is noteworthy that by incorporating certain antioxidants into, e.g., hamburger patties, their production is greatly reduced (64). If they are produced and ingested, then it may be appropriate to consider dietary anti-mutagens (65). These have a number of mechanisms, including enhancing excretion, reducing cellular uptake, and modifying xenobiotic metabolism, among other mechanisms (14). For example, Kada et al. (51) used mostly bacterial mutagenicity test systems, and screened large numbers of plant extracts for anti-mutagenicity.

Dietary fibre may also be one of the most effective approaches to reduction of mutagenicity. We have previously commented on the confusion in the literature, which we interpret as being caused by current chemical definitions of dietary fibre allowing a diverse group of chemicals, with considerably different properties (66,67). In an experimental model, we have shown that wheat bran may be one of the most effective sources of dietary fibre to protect against mutation and cancer (68,69). In rats fed a high fat AIN–76 (defined) diet in...
which 10% of the cornstarch was substituted with 10% wheat bran, as compared with controls fed a high fat AIN-76 (defined) diet with no wheat bran, the presence of this dietary fibre source reduced the bioavailability of the carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) gavaged to the rats. The presence of the wheat bran led to a reduced colonic transit time, and reduced bioavailability of the dietary carcinogen (IQ), by enhanced IQ excretion, reduced IQ metabolism to a reactive metabolite, and reduced amount of IQ in the plasma.

Examples of Mechanisms of Anti-mutagen Protection against Different Classes of Chemicals

Anti-mutagenic effects of mutagen scavenging by chlorophyllin and related molecules: Seminal work by Hayatsu and coworkers showed that hemin, chlorophyll and related molecules reduced the mutagenicity of various mutagens (70,71). Arimoto and coworkers (70) used highly purified samples of chlorophyllin (a stabilised form of chlorophyll) and related compounds, in order to establish the mechanism of the inhibition. They recognised that mutagens with polycyclic planar structures were those most likely to be inhibited, and therefore suggested a mechanism of trapping of the mutagens by chlorophyllin through complex formation at the planar surfaces of these molecules. They explored this possibility, by considering covalently linked chlorophyllin as a ligand, and measuring the adsorption of mutagens to this ligand. Their data showed that most of the compounds that contained three or more fused rings were very strongly adsorbed, with high binding affinities, whereas those having one ring or two fused rings were only poorly adsorbed. They concluded that trapping by complex formation plays a mechanistic role in the anti-mutagenic actions of chlorophyllin against many mutagens, including the frameshift mutagen, quinacrine, the fungal toxin, aflatoxin B1, and several heterocyclic amines.

Anti-mutagenic effects of modifying xenobiotic metabolism: Most (but not all) mutagens interact with DNA, and that step usually requires metabolic activation, by one of several kinds of cytochrome P450s (CYPs) (72). Various types of anti-mutagen appear to act through inhibiting induction of these enzymes, or otherwise interfering with the activation process. For example, black tea polyphenol (theaflavin) has been shown to be able to reduce the induction of CYP1A1, at least in a human cultured cell line, and this has been suggested as one mechanism by which this class of chemicals can reduce DNA damage, and possibly also reduce cancer risk (55). The activation of specific groups of mutagens resides with other specific classes of enzymes. For example, acetyl CoA:arylamine N-acetyltransferase (NAT; E.C. 2.3.1.5) enzymes play a key role in the metabolic activation of aromatic amine and aromatic nitro mutagens to reactive electrophilic intermediates (73).

While reducing the induction of various CYPs may be beneficial, there are also several classes of detoxifying enzymes whose direct induction may help to reduce the available levels of mutagen. The transcription of many detoxifying enzymes is regulated through the antioxidant response element (ARE), whose transcription factor, Nrf2, is repressed under basal conditions by Keap1 (24,74,75). Dietz et al., 2008, described how extracts from the roots of Angelica sinesis, induced the detoxification enzyme NAD(P)H: quinone oxidoreductase 1 (NQO1) by alkylating Keap1. They demonstrated that lipophilic extracts, containing ligustilide, and monooxygenated ligustilide, alkylated some key cysteine residues in the human Keap1 protein, thereby activating the Nrf2 gene, and upregulating the transcription of ARE-regulated genes (76). Lee and co-workers also showed that lignans from the fruit of Schisandra chinensis led to a similar endpoint through nuclear accumulation of Nrf2 (77). A wide range of phytochemicals and other putative chemopreventive agents have been found to be anti-mutagens through similar mechanisms (17).

Anti-mutagenic effects of modifying cellular transport of a mutagen: Another mechanism by which humans or animals are protected from xenobiotics is through these being actively extruded from cells by the action of one or more of a series of ATP-binding cassette drug transporter proteins (78). Those that play a key role in humans are the multidrug resistance proteins (P-glycoproteins, encoded by the MDR1 (ATBC1) and MDR3 (ATBC3) genes), multidrug resistance associated proteins (MRP1–7) and the breast cancer resistance protein (BCRP). The proteins have similar but distinct cellular locations, and substrate specificities. They jointly determine whether a given mutagen will enter various tissues, including the gastrointestinal tract, lung, liver, brain, testis, ovaries and foetus. By these means, they modify the absorption, distribution and excretion of mutagens, their metabolites and conjugates. In general (but not always), the net effect is to prevent or reduce mutagenesis or carcinogenesis.

Anti-mutagenic effects of micronutrient supplementation: It has been recognized for many years that micronutrient deficiencies can lead to effects on DNA or the chromosome comparable to ionising radiation (2,3). There is thus a considerable literature suggesting that micronutrient optimization or even supplementation may protect against mutation and cancer (79–81). In these studies, however, it is of considerable importance to recognise that there is always an optimal range of micronutrients, and this optimum may vary among individuals (82). There is thus a considerable justifica-
tion for knowing the starting concentration of micronutrients before recommending supplementation, and optimising rather than merely supplementing (82). Providing it is carefully monitored and regulated, optimisation of levels of nutrients and micronutrients must be considered an important mechanism of anti-mutagenesis.

Effects of Anti-mutagens in Human Populations

Epidemiological data suggests that it is possible to prevent cancer and other chronic diseases. While the most obvious mechanisms involve avoiding exposure to recognized risk factors, many of these risk factors are not always obvious and may be difficult, if not impossible, to avoid. An alternative approach is through antimutagenic mechanisms, which protect the individual against DNA damage, oxidative stress, and chronic inflammation (83). There is reason to believe that it is possible to render the organism more resistant to mutagens and/or carcinogens, by administering chemopreventive agents which will include, but are not restricted to, anti-mutagens.

Primary prevention will be aimed at apparently healthy individuals, whereby triggering mutation-protective mechanisms, either in the extracellular environment or inside cells, leads to the reduction or prevention of mutation and cancer initiation (14). Mechanisms that have been suggested for this involve modifying transmembrane transport, modulating metabolism, blocking reactive species, inhibiting cell replication, maintaining DNA structure, modulating DNA metabolism and repair, and controlling gene expression. While this approach is theoretically possible, it has only rarely been demonstrated to be efficacious in humans.

Aflatoxin B1 is a known dietary mutagen and carcinogen, associated with food spoilage (71,84). Not only does it show strongly positive mutagenic effects in various experimental models, aflatoxin B1 is a potent mutagen and carcinogen in humans (84). It was one of the molecules against which chlorophyllin was shown to be efficacious, at least in vitro and in animal models (70). An important question is: “Can the human population be protected from Aflatoxin B1 mutagenesis by an antimutagen such as chlorophyllin?” This question was asked by Kensler, Groopman and co-workers (85), in their studies in Qidong, China. In particular, they had been depending upon animal data for the idea that a specific anti-mutagen could reduce the mutagenicity and carcinogenicity of aflatoxin B1 in humans. They developed and validated non-invasive biomarkers of aflatoxin B1 exposure in the rat model (19). Their studies showed that most of the aflatoxin B1 absorbed in one day was excreted as metabolites in the urine, and a specific aflatoxin B1-N7-guanyl DNA adduct, is trans-ported into the urine within a few hours of exposure. The level of this urinary biomarker in rats correlated with a reduction in the levels of aflatoxin B1-DNA adducts in the rat liver. Their subsequent studies sought an answer to the question—how do you move from a rodent, which you can sacrifice, to a human? The urinary biomarker of aflatoxin B1 exposure was used to assess the effects of a stabilised form of chlorophyllin intervention in aflatoxin B1-exposed human populations, to suggest a highly successful human intervention (84). This was an important step in establishing the prime importance of this field in protecting against mutagenesis in humans. Since this time, stabilised chlorophyllin has been accepted as a very important human anti-mutagen, and dietary supplement. Perhaps more importantly, it has become recognised that anti-mutagenesis may be an important step in the battle to prevention of carcinogen-induced cancer in humans.

Antimutagenesis Studies: Where Are They Heading?

The Aflatoxin B1-chlorophyllin example identified above is a success story in human anti-mutagenesis (85). However, there are some notorious failures. For example, there is currently a considerable debate as to whether selenium intervention is beneficial, either in mutation and cancer prevention or during cancer therapy (86). I suggest that there are at least three important steps to be taken in future studies:

- Evaluation of key sources of mutation in a given population before adding antimutagens: The selenium example is one where this supplement was given to a population that was not actually deficient in it, leading to variable results and considerable controversy (86).
- Mechanism-based identification of specific antimutagens for specific purposes: A number of antimutagens have a range of effects, some positive and some negative (87).
- More sensitive biomarkers for human studies: It is not sufficient to just understand the levels of a nutrient, e.g., selenium in toenail clippings (88), but to understand whether regulation of this level will beneficially affect genomic stability (80,82).

These approaches will lead to evidence-based antimutagenesis, and enable this field to establish its true role in protection against chronic diseases in humans.

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