localized thinning and to meet the component drawing requirements.

Photograph of one component namely the combustion chamber cap and the various stages involved in forming the same on a hydraulic press is shown in Figure 13.

In addition to saving in capital expenditure, the above action has resulted in substantial cost saving in F.E. as otherwise these components should have been procured from the US. Computation of cost saving as well as saving in F.E. is given in Table 9.

REVIEW ARTICLE

Protection by caffeine against oxic radiation damage and chemical carcinogens: Mechanistic considerations

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There is little doubt that caffeine administered after exposure to UV light enhances the damage to cells and organisms by inhibiting photoreactivation, excision and/or recombinational repair. However, when already present in the system, it affords remarkable protection not only against O_2-dependent component of radiation damage, but also against chemical carcinogens that require metabolic activation. I discuss here possible mechanistic aspects briefly.

Early experiments

A little over 40 years ago, Witkin[1] demonstrated that post-treatment with caffeine reduces the survival, but dramatically increases the mutation frequency of UV-irradiated *Escherichia coli* cells. Between then and the early seventies, several papers[1-8] dealt with potentiation of the UV-induced damage in a variety of prokaryotic and eukaryotic cells and also suggested that caffeine possibly inhibits photoreactivation, excision repair and also the recombinational repair. It had been well known by then that the photoproducts of UV radiation largely consist of cyclobutane type of thymine–thymine dimers than cytosine–cytosine or thymine–cytosine dimers. From mechanistic point of view, scores of these papers allow us to state that in order to potentiate, caffeine must be administered *immediately after* the treatments with UV; pre-treatment with caffeine exerts no discernible effect. However, in marked contrast to the damage induced by UV, that induced by X-rays in mouse L^5, *E. coli*[9] or Ehrlich ascites tumour[10] cells are not affected by post-treatment with caffeine. Several experiments with *Secale cereale* and *Vicia faba* conducted in Warsaw[11,12] and Uppsala (Sweden)[13,14] failed to demonstrate the potentiating action of caffeine on chromosomal aberrations induced by X-rays. Similarly, caffeine post-treatment did not enhance the *X-ray-induced chromosomal aberrations in Chinese* hamster cells[14].

Two papers of particular interest are those of Yamamoto and Yamaguchi[15] and Ahnström and Natarajan[16]. Both these groups had used barley seeds as test system, and gamma rays as the radiation. Towards the end of this review, it would be evident that an insight into mechanistic aspects has been gained largely with the help of barley seeds exposed to ^{60}Co gamma rays. Yamamoto and Yamaguchi[15] concluded that caffeine increases the frequency of gamma ray-induced fragments by inhibiting the rejoining of chromatid breaks. Since caffeine was ineffective when added 30 min after irradiation, it was inferred that the rejoining process is completed within 30 min after the gamma irradiation. Ahnström and Natarajan[16] found that when barley seeds were exposed to gamma rays and then exposed to caffeine for the first 5 h of the germination period, the frequency of gamma ray-induced dicentrics and rings doubled. When the interval between irradiation and caffeine post-treatment exceeded 5 h, no enhancement of the radiation-induced aberration frequency was obtained.

A very significant observation[16], however, is that caffeine has no potentiating effect on the frequency of dicentries and rings when neutrons are used instead of gamma rays. This observation that caffeine potentiates the gamma ray but *not* neutron-induced damage in the barley seeds raised a question, about 20 years ago in

the mind of this author, on the possible relationship between the post-irradiation oxygen effect (class III damage of Powers[17]) and the modifying action of caffeine. Secondly, it was also kept in view that the DNA lesions induced by X-rays and gamma rays largely consist of single and double-strand breaks and, therefore, these ought *not* to be mixed up with the cyclobutane type of thymine–thymine dimers induced by the UV. Thirdly, it had been noted that more than 80% of these studies involved *post*-treatments with caffeine. The question that could not be answered was as to the effects of caffeine administered *just before* irradiation, so that its influence, if any, when present during the exposure of actively metabolizing cells and tissues to irradiation, could have been known. The reason for this consideration is that the radiation-induced free radicals in metabolizing systems are indeed very short-lived with lifetimes of the order of 10^{-9}–10^{-6} sec. Possible reactions of caffeine with these free radicals and their overall influence on survival, growth rate, chromosomal aberrations and mutations would be totally missed out in studies with caffeine administered *post*-irradiation, unless the test systems used are dry spores and seeds which are metabolically inert (see next chapter). Radiation-induced free radicals quite persist at room temperature in dry biological systems[17,19].

Caffeine and free radicals

The aim of the early investigations initiated at this School was simply to understand whether the reported potentiation of damage by caffeine is influenced by the magnitude of radiobiological oxygen effect. By 1970, there was no doubt at all that oxygen effect develops only because of the reaction of molecular oxygen with some fraction(s) of the radiation-induced free radicals. The earlier experiments of Powers and co-workers[17,18] in the dried spores of *Bacillus megaterium* and those of Caldecott *et al.*[19], Konzak *et al.*[20], Conger *et al.*[21] in dry barley seeds had shown elegant techniques by which the magnitude of post-irradiation O_2-dependent (oxic) and O_2-independent (anoxic) pathways of radiobiological damage could be differentially manipulated. For instance, dry barley seeds (seed moisture content equilibrated to ~3%) exposed to ^{60}Co gamma rays and then post-hydrated at ~2°C for 8–10 h in O_2-saturated water (distilled water degassed and O_2-bubbled for ~20 min at 10°C) develop 4–5-fold increased damage than those post-hydrated in O_2-free (degassed and N_2-saturated at 10°C) water. The O_2-and N_2-saturated water contain dissolved O_2-concentration of about 1.8×10^{-3} M and 1.0×10^{-6} M respectively. Ahnström and Mikaelsen[22] and Ahnström and Sanner[23] have shown the effect of such hydration on the rates of decay of radiation-induced radicals and oxygen-sensitive centres in barley seeds. The barley system is also ideal for computing seedling injury[24], chromosomal aberrations during early mitoses of shoot-tip meristems[20], peroxidase activity[25] and total peroxides[26]. The important role played by barley seed in the physicochemical characterization of radiobiological oxygen effect(s) has been reviewed[27].

Using barley seeds (3.6% moisture content) irradiated *in vacuo* (10^{-2} Torr) with gamma rays and then subjected to post-treatments such as heat shock (at 60°C for 90 sec), and oxygenated or oxygen-free post-hydration in the presence or absence of caffeine, Kesavan *et al.*[28] demonstrated that (i) caffeine affords significant radioprotection against oxic pathways, (ii) the magnitude of such protection decreases after a wet heat shock to the seeds, (iii) caffeine dramatically potentiates the anoxic component of radiation damage, and (iv) a combination of heat-shock and caffeine enhances the anoxic damage to almost the same level as that of oxic damage. Based on earlier studies[20,29,30], it was possible to postulate that caffeine competes with heat shock for the radiation-induced O_2-sensitive sites (free radicals then designated as A_n)[31]. Since the reaction of the O_2-sensitive sites with oxygen forms several oxidizing peroxy radical intermediates (e.g. superoxide, hydroperoxy radicals) and peroxides, it was postulated that radioprotection against oxic damage results from mutual annihilation of caffeine and these oxidants[28]. This postulate was supported by subsequent experiments performed with different approaches but towards the goal of its verification. All these studies[28,31–49] emphasized on the physiochemical reactions between caffeine and the radiation-induced oxidants (A_n) as the causal mechanism of radioprotection.

An emphasis is laid on the fact that caffeine affords significant radioprotection against oxic pathway in metabolizing seeds[37,39], aqueous suspension of bacterial spores[42,49], CHO cells[43], but in these cases, caffeine must be present during irradiation. The point is that in the actively metabolizing cells, the lifetimes of the radiation-induced sites are extremely shorter than in dry seeds.

The exact nature of the radiation-induced O_2-sensitive sites with which caffeine undergoes mutually annihilatory reaction was elucidated with the help of pulse radiolysis studies[42]. We showed that caffeine reacts with electrons (e^-_{aq}) and hydroxyl radicals (OH) at rate constants of $1.5 \times 10^{10}\ M^{-1}\ sec^{-1}$ and $6.9 \times 10^{9}\ M^{-1}\ sec^{-1}$ respectively. Under these circumstances, caffeine effectively competes with oxygen for electrons. Oxygen reacts with hydrogen radicals (H) (ref. 50) and electrons (e^-_{aq}) (ref. 51), at rate constants (k) of $1.2 \times 10^{10}\ M^{-1}\ sec^{-1}$ and $1.9 \times 10^{10}\ M^{-1}\ sec^{-1}$ respectively.

Radiation chemistry and damage

In actively metabolizing cells, there is considerable water besides the target molecules (e. g. DNA, membranes, etc.), denoted by RH_2. The 'direct' and 'indirect' actions of radiations would initiate radiation chemical events as follows

$$H_2O \sim\!\!\!\!\!\!\rightarrow OH^{\cdot}, H^{\cdot}, e^{-}_{aq} \quad (1)$$

$$RH_2 \sim\!\!\!\!\!\!\rightarrow RH^{\cdot}, H^{\cdot}, e^{-}(\text{trapped}). \quad (2)$$

Since there is oxygen normally present in metabolizing cells, it reacts with reducing species as follows:

$$H^{\cdot} + O_2 \longrightarrow HO^{\cdot}_2 \quad (3)$$

$k = 1.2 \times 10^{10}\ M^{-1} sec^{-1}$, (ref. 50)

$$e^{-}_{aq} + O_2 \longrightarrow O^{\cdot-}_2 \quad (4)$$

$k = 1.9 \times 10^{10}\ M^{-1} sec^{-1}$, (ref. 51)

$$RH^{\cdot} + O_2 \rightarrow R\bar{H}O^{\cdot}_2. \quad (5)$$

If $RH^{\cdot}$ is DNA radical, then the rate constant (k) is about $5 \times 10^7\ M^{-1} sec^{-1}$ (ref. 52). RHO_2 is the damaged target molecule.

The hydroperoxy radical ($HO^{\cdot}_2$) and superoxide anion ($O^{\cdot-}_2$) are known for the following reactions

$$HO^{\cdot}_2 + HO^{\cdot}_2 \longrightarrow H_2O_2 + O_2 \quad (6)$$

$k = 8.6 \times 10^5\ M^{-1}\ sec^{-1}$ (ref. 53).

The hydrogen radicals ($H^{\cdot}$) and hydroxyl radicals ($OH^{\cdot}$) may react with H_2O_2 as follows:

$$H^{\cdot} + H_2O_2 \longrightarrow H_2O + OH^{\cdot} \quad (7)$$

$k = 6.0 \times 10^7\ M^{-1}\ sec^{-1}$ (ref. 50)

$$OH^{\cdot} + H_2O_2 \longrightarrow H_2O + HO^{\cdot}_2 \quad (8)$$

$k = 4.5 \times 10^7\ M^{-1}\ sec^{-1}$ (ref. 54)

$$HO^{\cdot}_2 + OH^{\cdot} \longrightarrow H_2O + O_2 \quad (9)$$

$k = 2.5 \times 10^9\ M^{-1}\ sec^{-1}$ (ref. 55).

$OH^{\cdot}$ may recombine to yield more H_2O_2 as follows:

$$OH^{\cdot} + OH^{\cdot} \longrightarrow H_2O_2 \quad (10)$$

$k = 5.5 \times 10^9\ M^{-1}\ sec^{-1}$ (ref. 54).

The superoxide anion radicals ($O^{\cdot-}_2$) formed by reaction 4 could also be produced by deprotonation of $HO^{\cdot}_2$, depending upon pH.

$$HO_2 \xrightarrow{\quad} H^{+} \quad O^{\cdot-}_2 \quad \text{(ref. 56)} \quad (11)$$

$HO^{\cdot}_2$ and $O^{\cdot-}_2$ could react as follows:

$$HO^{\cdot}_2 + O^{\cdot-}_2 \longrightarrow O_2 + HO^{-}_2 \xrightarrow{H_2O} O_2 + H_2O_2 + OH^{-} \text{ (ref. 57).} \quad (12)$$

The abovesaid reactions account for enhanced radiobiological damage to the target molecule, RH_2.

Reaction 5 describes the formation of $RHO^{\cdot}_2$ which can be terminated by agents (e.g. cysteine) capable of donating H-atom as follows:

$$RHO^{\cdot}_2 + XSH \longrightarrow RHOOH + XS^{\cdot}. \quad (13)$$

RHOOH is, however, *not* the original RH_2, and it represents possibly damaged state. Alternatively, 'chemical repair' leading to restoration of RH_2 can also take place as follows:

$$RHO^{\cdot}_2 + XSH \longrightarrow RH_2 + XS^{\cdot} + O_2. \quad (14)$$

The other pathway of damage to target molecule (RH_2) could be owing to sequential attack by $OH^{\cdot}$ and H_2O_2 as follows:

$$RH_2 + OH^{\cdot} \longrightarrow RH^{\cdot} + H_2O \quad (15)$$

$$RH^{\cdot} + H_2O_2 \longrightarrow R^{*} + OH^{\cdot} + OH^{-} + H^{+}. \quad (16)$$

R* represents the lethally and irreversibly damaged target molecule. These two reactions representing double oxidation scheme were suggested in a different context by Powers[58]. In my opinion, it is generally applicable, as the $OH^{\cdot}$-scavengers do protect spores, seeds and mammalian cells exposed to gamme rays[49]. The basic consideration here is that the solvated electron (e^{-}_{aq}) resulting from radiolysis of water is possibly protective, especially in aerobic systems, as follows:

$$OH^{\cdot} + e^{-}_{aq} \longrightarrow OH^{-} \quad (17)$$

$k = 3.0 \times 10^{10}\ M^{-1}\ sec^{-1}$ (ref. 58)

$$H_2O_2 + e^{-}_{aq} \longrightarrow OH^{\cdot} + OH^{-} \quad (18)$$

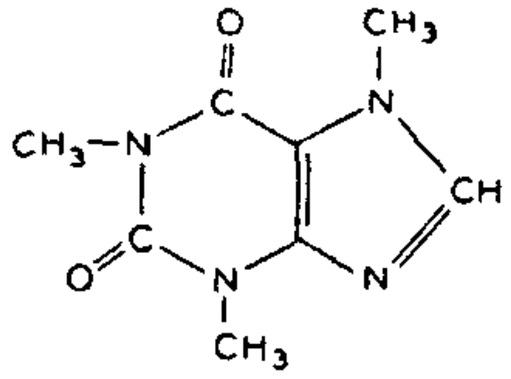
$k = 1.2 \times 10^{10}\ M^{-1}\ sec^{-1}$ (ref. 58).

Hence, e^{-}_{aq} which reduces both $OH^{\cdot}$ and H_2O_2 and thereby protects the target molecule (RH_2) against the damaging reactions 15 and 16 is a protective agent. The molecular oxygen reacts with e^{-}_{aq} (reaction 4) not only to form harmful superoxide anion ($O^{\cdot-}_2$), but also to curtail the protective reactions 17 and 18.

Anaerobic irradiation results in greatly diminished damage. All the oxygen-dependent reactions which are indeed harmful are eliminated. The hydroxyl radicals are largely quenched by e^{-}_{aq} and $H^{\cdot}$ to form OH^{-} and H_2O. Some damage by $OH^{\cdot}$ (reactions 15 and 16) is, however, likely. H_2O_2 is formed by reaction 10.

Caffeine on radiation-induced chemical events in O_2 and N_2

An examination of the structure of caffeine (caf) suggests that it could readily accept electrons. In fact, it has been demonstrated that caffeine reacts at a rate constant (k) of $1.5 \times 10^{10}\ M^{-1}\ sec^{-1}$ with electrons. Further, caffeine scavenges hydroxyl radicals ($OH^{\cdot}$) at a rate constant (k) of $6.9 \times 10^{9}\ M^{-1}\ sec^{-1}$.



1,3,7-trimethyl xanthine or caffeine

When present *during* irradiation, caffeine molecules could be visualized to undergo degradation by both 'direct' and 'indirect' actions of radiations as follows:

$$Caf \xrightarrow{\text{Direct action}} Caf^{\cdot} + e^{-} \quad (19)$$

$$Caf + OH^{\cdot} \xrightarrow{\text{Indirect action}} Caf^{\cdot} + OH^{-} \quad (20)$$

In this scheme, the hydroxyl radicals are derived from radiolysis of water (reaction 1).

Let us now examine the mechanism by which caffeine causes radioprotection in oxygenated but radiosensitization in oxygen-free situations in the biological systems. First of all, it should be kept in view that caffeine *only* potentiates the radiation-induced damage in the *absence* of oxygen. In fact, a certain minimum concentration of oxygen is absolutely essential for caffeine to be radioprotective. In this regard, the recent demonstration by Kesavan *et al.*[47] that caffeine acts as a radioprotector, or a radiosensitizer or neither depending upon the concentration of oxygen is quite relevant. In this study, the O_2-concentration in the post-hydration medium (OCHG) was adjusted at 0%, 10%, 30%, 50%, 80% and 100% corresponding to 1×10^{-6}, 1×10^{-4}, 5×10^{-4}, 9×10^{-4}, 1.4×10^{-3} and 1.8×10^{-3} M of oxygen respectively. Caffeine potentiates the post-irradiation damage at OCHG of $\leq 30\%$, exerts no influeuce at OCHG of $\sim 50\%$ and affords radioprotection at OCHG of $\geq 80\%$.

These data possibly suggest that protection results from competition between caffeine and oxygen for radiation-induced caffeine- and oxygen-sensitive species. It is now known[42] that both caffeine and oxygen react equally well with electrons and also possibly with the lesions ($RH^{\cdot}$) resulting from the target molecule (RH_2). Based on this, the following reactions are postulated:

$$Caf + e^{-} \longrightarrow Caf^{-} \quad (21)$$

$$Caf^{\cdot} + e^{-} \longrightarrow Caf^{=} \quad (22)$$

These two reactions could possibly diminish the scope for harmful reactions of electrons with O_2 (reaction 4) to result in increased formation of oxidizing species. Further $Caf^{\cdot}$ can react also with O_2 as follows:

$$Caf^{\cdot} + O_2^{-} \longrightarrow Caf + O_2 \quad (23)$$

Should O_2^{-} be harmful to the cellular targets, this reaction is also possibly protective.

Further, the scavenging of $OH^{\cdot}$ by caffeine (reaction 20) could by itself be protective as the magnitude of $OH^{\cdot}$-mediated damage to the target molecule (RH_2) (reactions 15 and 16) would be greatly diminished. Production of hydrogen peroxide by recombinations among $OH^{\cdot}$ (reaction 10) would also be greatly reduced. In addition, caffeine could also render $HO_2^{\cdot}$ harmless as follows:

$$Caf + HO_2^{\cdot} \longrightarrow Caf^{\cdot} + HO_2^{-}. \quad (24)$$

$Caf^{\cdot}$ resulting from a number of different pathways (reactions 19, 20, 24) can react with oxygen as follows:

$$Caf^{\cdot} + O_2 \longrightarrow Caf\,O_2^{\cdot} \quad (25)$$

The overall effect would again be a reduction in the availability of O_2 for deleterious reactions. TThere is expected a consequential protection. Recently, there has been an unexpected finding[59] that post-treatment with caffeine affords significant protection against radiation-induced chromosomal aberrations in the bone marrow of mice given 1.5 Gy of ^{60}Co gamma rays. Since electrons, H-atoms and hedroxyl radicals are unlikely to persist in mice for several minutes until post-treatment, the possibility that caffeine reacts with more stable peroxides needs to be considered. For instance, the DNA peroxy radical ($RHO_2^{\cdot}$) formed by reaction 5 is possibly less reactive (i.e. more stable) than $RH^{\cdot}$. Singh and Kesavan[48] suggested that caffeine by virtue of its binding with denatured regions of the DNA could bring about restoration of the target molecule as follows:

$$RHO_2^{\cdot} + Caf \longrightarrow Caf^{\cdot} + RH_2 + O_2 \quad (26)$$

(restoration)

It is postulated here that H-atom from the 8th position of CH-group could be abstracted by oxidizing agents. More studies are required to verify this highly tentative postulate.

These aforesaid reactions possibly occur in well-oxygenated, caffeine-containing biological systems exposed to gamma rays. The dry seeds irradiated *in vacuo* and then exposed to caffeine during oxic post-hydration at $\sim 2^{\circ}C$ for 8 h also register radioprotection against

oxic pathway of damage. This is because the radiation-induced O_2-sensitive and O_2-insensitive free radicals have considerably longer lifetimes in dry seeds[21]. The electron spin resonance (ESR) studies being carried out in our laboratory reveal that the gamma ray-induced free radicals in the dry seeds remain stable for several hours at room temperature. Removal of electrons in competition with oxygen (reactions 21 and 22) could be the initial chemical events underlying the caffeine-mediated radioprotection.

In the absence of oxygen, caffeine significantly potentiates the radiobiological damage. Although experimental evidence is not strong, there are many who believe that anoxic radiation damage largely results from hydroxyl radicals[42,49,58]. Since the removal of hydroxyl radicals by caffeine could explain only a protective but *not* a sensitizing action, many interpretations take into account the two following aspects[46,49].

1. Removal of electrons by caffeine, in the absence of oxygen, diminishes the scope for harmless recombinations of hydroxyl radicals (reactions 17 and 18). The 'chemical repair' requires electrons and hydrogen atoms[60].
2. Since caffeine binds to denatured regions of the DNA[4,61] it has been postulated that the repair enzymes are possibly not effective in recognizing and excising the defective sites[42,49].

The most significant revelation from an applied standpoint is that caffeine in total anoxia or extreme hypoxia acts only as a sensitizer[28,31–48]. At an oxygen-concentration of 9×10^{-4} M, caffeine is neither a sensitizer, nor a protector. Caffeine becomes an effective radioprotector when the O_2-concentration is about 1.4×10^{-3} M and above; it is a radiosensitizer at or below an O_2-concentration of 5.0×10^{-4} M (ref. 47). What is indeed promising is that all aerobic systems normally contain around 1.0×10^{-3} M of oxygen, but the deep-seated tumour cells are indeed hypoxic. A combination treatment with caffeine and radiation might help not only in reducing damage to normal *cells, but also in preferentially enhancing damage to the* hypoxic tumour cells in cancer therapy.

Caffeine and environmental carcinogens

Background information

Some thirty-eight years ago, Miller and Miller[62] reported that aminoazo dyes require metabolic activation before these become carcinogenic. Today, this is known to be true for many of the chemical carcinogens found in the environment[63]. Activation of such precarcinogens *in vivo* generally occurs in the microsomal cell fraction and involves a series of biochemical reduction–oxidation processes catalysed by mixed-function oxidases[60]. The 'activated' carcinogen is, in general, a very reactive electrophilic species or a free radical.

The other aspect to be kept in view is that cancer is a multi-step process, involving at least two stages recognized as initiation and promotion. Initiation represents an irreversible alteration of the cellular DNA that could lead to carcinogenic transformation of the cell. Promotion produces conditions that allow the initiated cell to become clonally unstable so that it actually produces a tumour. Further, superoxide (O_2^-) and other oxy-radicals are known to be involved in promotion[64]. Since promotion is reversible, there is reason to believe that the use of antioxidants and other strategies that control free radical reactions can protect initiated cells against promotion and, thus, prevent the ultimate development of a tumour.

Since caffeine and its analogues scavenge oxy-radicals, there is reason to expect them to afford protection against chemical carcinogens that require metabolic activation. When we consider the cytochrome P-450-catalysed microsomal metabolism of chemical carcinogens, it is evident that these processes utilize oxygen in catalysing redox reactions with carcinogens and generate activated oxygen products (O_2^-, $OH^{\cdot}$, $HO_2^{\cdot}$ *and* H_2O_2) *and toxic metabolites* (phenols, diols, dihydrodiols and epoxides) capable of reacting with cellular target nucleophiles[65]. It is, therefore, not surprising that, in 1974, Rothwell[66] had indeed shown that caffeine exerts a dose-related inhibitory effect on the carcinogenic action of cigarette-smoke condensate [polynuclear aromatic hydrocarbons (PAH)] in mouse skin. This finding had indeed been preceded by a report[67] that, activated carcinogens are formed in thermal processes involving free radical species occurring as a result of combustion in the cigarette smoke. More recently, Pryor[68] has demonstrated the involvement of free radical reactions in cigarette smoke carcinogenesis. It is interesting that the cigarette tar has a stable ESR signal, and when the tar radical and DNA *are incubated* together, an *ESR* signal appears in the reisolated DNA. This long-lived free radical is a semiquinone ($QH^{\cdot}$), which is partially water soluble. Further, the tar radical associates with DNA. The tar semiquinone reduces dioxygen to form the superoxide radical (O_2^-), which then produces hydrogen peroxide (H_2O_2). And lastly, tar (possibly through its phenolic functionalities) chelates metal ions such as iron or copper. The association of tar with DNA greatly enhances the damage-producing process. This is because the hydroxyl radicals ($OH^{\cdot}$), resulting from reduction of H_2O_2 by chelating metals, are extremely short-lived, with a half-life of only 10^{-9} sec; further, these can only diffuse 10-30 molecular diameter before

reactions[68]. The oxidation of PAH to hydroquinones (QH_2) and quinones (Q) is thought to involve radical reactions. The sequence of steps involved in the generation of oxy-radicals, particularly, the most damaging $OH^{\cdot}$ from the PAH (ref. 68) is shown as follows:

$$PAH \xrightarrow{\text{enzymic oxidation}} \text{Epoxides, hydroquinones } (QH_2) \text{ and quinones (Q)} \quad (27)$$

$$QH_2 \xrightarrow{\text{spontaneous oxidation}} QH^{\cdot} \quad (28)$$

$$Q \xrightarrow{\text{enzymic reduction}} QH_2 \quad (29)$$

$$\rightarrow QH^{\cdot} \quad (30)$$

$$QH^{\cdot} + O_2 \longrightarrow Q + O_2^{\cdot-} + H^+ \quad (31)$$

$$2O_2^{\cdot-} + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (32)$$

$$H_2O_2 + Fe^{2+} \longrightarrow HO^- + HO^{\cdot} + Fe^{3+} \quad (33)$$

The hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide possibly damage the DNA (RH_2) through the reactions 15 and 16 already described in the context of ionizing radiations. In fact, it would seem that ionizing radiation, bleomycin, adriamycin, cigarette tar and several others produce superoxide ($O_2^{\cdot-}$), and a cascade of activated oxygen species which ultimately lead to generation of hydroxyl radicals[68]. The $OH^{\cdot}$ then is able to both abstract hydrogen atoms from DNA (e.g. converting thymine to hydroxymethyl uracil) and add to DNA (e.g. producing thymine glycol).

Mitomycin C can be activated to a semiquinone ($QH^{\cdot}$) by a one-electron process or to a quinone (Q) by a two-electron process[68]; further the mitomycin C semiquinone may bind to DNA by a free radical mechanism. In this context, it is relevant to refer to the observations of Abraham[69]. It was found that mice which were administered standard coffee by gavage are dramatically protected against genotoxicity of mitomycin C. The genotoxicity was assessed in the bone-marrow micronucleus test.

More recently, Abraham[70] reported that standard instant coffee affords significant protection against *in vivo* genotoxicity of 7, 12-dimethylbenz(a)anthracene (DMBA), benezo(a)pyrene (BP), aflatoxin B_1 (AFB_1) and urethane (UR). It is noted that all these carcinogens require metabolic activation. A survey of the literature[69-76] shows that in the very recent years, there has been increased recognition of the protective role of caffeine and caffeine-containing beverages against carcinogenesis by environmental chemicals.

Mechanism(s) of protection by caffeine

It is evident that the authors who all have reported on the protective action of caffeine (or coffee) against a variety of indirectly acting chemical carcinogens have not discussed the probable mechanisms in a crisp manner. There is practically no reference to the possible reaction of caffeine with electrons and hydroxyl radicals. Much of the interpretation has centred around an observation[71,77] that, addition of green coffee beans to the diet of mice and rats enhances the activity of glutathione S-transferase, an enzyme involved in the process of detoxification.

Since caffeine competes with oxygen for electrons[42], its overall influence on what is referred to as 'redox cycling' of quinones[78] should receive attention. As stated earlier, in this process, the quinone is reduced, or the hydroquinone is oxidized to a semiquinone, which, in turn, reduces oxygen to produce superoxide. Further, chemical reactions (32 and 33) finally result in the production of hydroxyl radicals.

Since caffeine effectively scavenges the hydroxyl radicals[42], it is likely to act as a protector, where hydroxyl radicals are involved in carcinogenesis. This is indeed so with most indirectly acting chemical carcinogens against which caffeine is now known to be protective[66,69-76]. The fact that hydroxyl radicals promote the carcinogen (DMBA)-DNA binding, and that ethanol, a good $OH^{\cdot}$-scavenger, inhibits such covalent binding[65], supports my contention that the protective action of caffeine against DMBA-mediated clastogenesis[70] possibly operates *via* $OH^{\cdot}$-scavenging. Similarly, caffeine's protective action against cigarette-smoke condensate carcinogenesis could reasonably be attributed to its $OH^{\cdot}$-scavenging action[26,79].

In conclusion, it may be stated that caffeine which once had been condemned as an inhibitor of DNA repair and hence a harmful agent, is fast emerging as an effective protector against several physical and chemical carcinogens that act *via* generation of oxy-radicals. Reasonable daily intake of caffeine-containing beverages is expected to be beneficial in view of radical reactions *in vivo*[80,81].

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