Stimulatory Effects of Sulfur and Nitrogen Oxides on Carcinogen Activation in Human Polymorphonuclear Leukocytes

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The occurrence of inflammatory processes and of cancer in the human respiratory tract is intimately associated. One of the major factors in this is probably the recruitment of and stimulated activity of polymorphonuclear leukocytes (PML) in conjunction with the ability of these cells to convert various carcinogens to their ultimate active metabolites. In this study, we demonstrate that nitrite and sulfite, the major dissolution products of the environmental pollutants nitrogen dioxide and sulfur dioxide in water enhance the metabolic activation of *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP-7,8-dihydrodiol), the proximal carcinogen of benzo[*a*]pyrene, to *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) and tetraols, the corresponding hydrolysis products, in human PML prestimulated with 12-*O*-tetradecanoylphorbol-13-acetate. Nitrite was more efficient than sulfite in stimulating the formation of reactive intermediates of BP-7,8-dihydrodiol in PML that covalently bind to extracellular DNA and, in particular, to intracellular proteins. The mechanism by which sulfite stimulates the metabolism of BP-7,8-dihydrodiol most probably involves the intermediate formation of a sulfur trioxide radical anion ($SO_3^{\cdot-}$)the subsequent formation of the corresponding sulfur peroxyl radical anion ($^{\cdot}OOSO_3^-$) in the presence of oxygen. The mechanism underlying the stimulatory action of nitrite is less clear but the major pathway seems to involve myeloperoxidase. These results offer an explanation for the increased incidence of lung cancer in cigarette smokers living in urban areas. The major glutathione transferase (GST) isoenzyme in human PML is GST P1-1, a Pi-class form. The GST activity of PML was found to be inversely correlated with the extent of binding of BP-7,8-dihydrodiol products to exogenous DNA. These results suggest that individuals exhibiting high GST-activity in the PML may be better protected against the type of carcinogenic dealt with in this study. — Environ Health Perspect 102(Suppl 4):161–164 (1994).

Key words: activation, BP-7,8-dihydrodiol, human leukocytes, nitrogen oxide, sulfur oxide

Introduction

The exposure of airborne contaminants to man is extensive and many compounds are proven to be toxic and cause acute or more long-term effects. The most important source of harmful substances is cigarette smoke and epidemiological studies have clearly shown a close correlation between extent of smoking and primary cancer of the respiratory tract (*1–3*). The ultimate carcinogenic component or components in cigarette smoke has not been identified but it is likely that polycyclic aromatic hydrocarbons (PAHs) are important factors (*4*). Another source of PAHs is urban air. PAHs are produced by various combustion processes, and they are thus widely distributed contaminants (*5*).

The ultimate carcinogenic forms of PAHs are called bay-region diol epoxides (*6,7*). For instance benzo[*a*]pyrene, which is an important and the most studied PAH, is metabolized to *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP-7,8-dihydrodiol) by the sequential action of cytochrome P450 and epoxide hydrolase followed by epoxidation at the 9,10-position to yield *trans*-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE). The last step has been demonstrated to be carried out by cytochrome P450 (*6*), lipooxygenase (*8*), peroxidase (*9*), and pathways dependent on formation of peroxyl radicals (*9,10*). Other important contaminants in urban air and in cigarette smoke are sulfur dioxide (SO_2) and nitrogen dioxide (NO_2). These pollutants are harmful to the lung. Sulfur dioxide is known to cause bronchoconstriction especially in asthmatics. Nitrogen dioxide is known to cause direct damage to various lung cells and have, for instance, been shown to cause increased airway reactivity in asthmatics, a decrease of the respiratory host defense system and, probably, emphysema (*11,12*). In addition, these oxides may be cocarcinogenic with PAHs by enhancing, for instance, their metabolism and ultimate activation to reactive intermediates (*3*).

Both SO_2 and NO_2 dissolves into mucous and into the epithelial lining fluid and are readily hydrolyzed (*13*). In fact, the major products detected in blood and urine are sulfite, sulfate, nitrite, and nitrate, and it is possible that many of the effects, including the cocarcinogenicity, can be attributed to these metabolites rather than to SO_2 and NO_2 themselves (*13,14*).

Cigarette smokers also are more afflicted by inflammation, bronchitis, and emphysema than nonsmokers. Moreover, smokers living in urban areas seem to have an increased risk of developing lung cancer (*1*). One reason may be the simultaneous exposure of carcinogens in the smoke and atmospheric contaminants such as sulfur oxides and nitrogen oxides.

Inflammatory processes result in mobilization, accumulation, and infiltration of polymorphonuclear leukocytes (PML) at the afflicted site, and thus they release proteolytic enzymes and produce reactive oxygen

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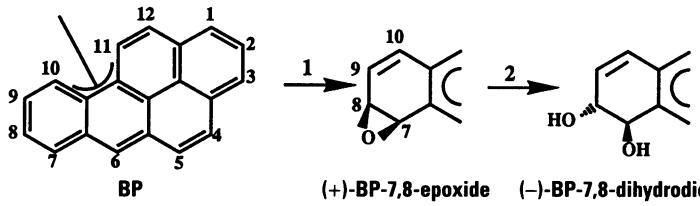

Figure 1. Metabolism of benzo[*a*]pyrene to (–) *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene via intermediate formation of (+)-BP-7,8-epoxide. Step 1 is usually catalyzed by cytochrome P450. Step 2 is catalyzed by epoxide hydrolase.

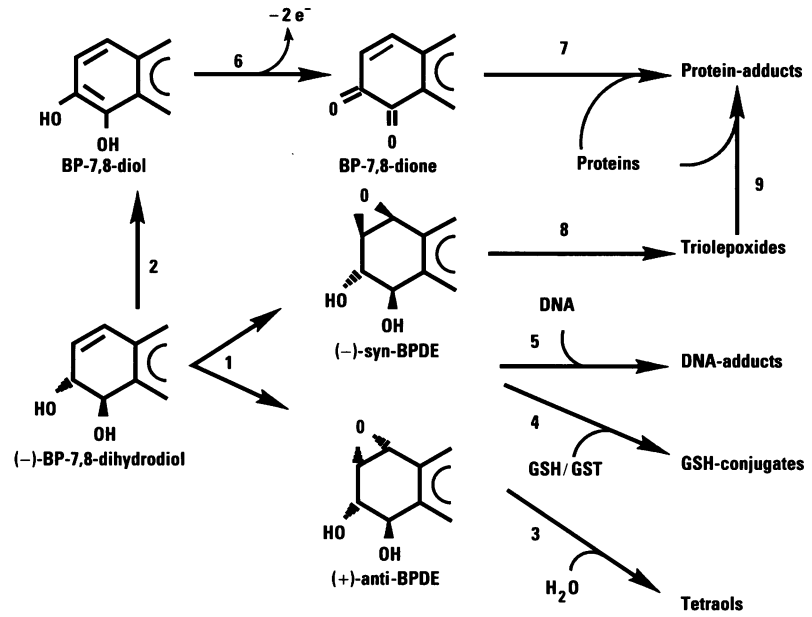

Figure 2. Metabolism of (–)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (BP-7,8-dihydrodiol) to various products. Alternative pathways are indicated by numbers and represents 1) the formation of *anti*- and *syn*-10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) diastereomers (dependent on cytochrome P450 or peroxidative mechanisms), 2) dehydrogenation of the 7,8-dihydrodiol to the corresponding 7,8-diol, 3) the spontaneous hydrolysis of BPDE to tetraols, 4) the glutathione transferase-catalyzed conjugation of BPDE with glutathione, 5) the reaction of BPDE with DNA, 6) the oxidation of BP-7,8-diol to the corresponding dione, 7) the reaction of the 7,8-dione with protein nucleophiles, 8) further activation of BPDE by hydroxylation at the 1 or 3 position to triolepoxides, and 9) these intermediate reactions with proteins.

intermediates. These include superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$). They may, in addition to fulfilling the protective aim, directly or indirectly disturb normal cellular activities in adjacent tissues by, for instance, initiating lipid peroxidation, inhibition of essential metabolic pathways, or destruction of DNA. In addition, prolonged inflammation may lead to tissue alterations such as hyperplasia and fibrosis, alterations that may render the tissue more susceptible to tumor development. PML also have the capacity to activate carcinogens to their ultimate forms. For instance, these cells activate BP-7,8-dihydrodiol, the proximal carcinogen of BP to BPDE (Figure 1). This property of PML may be of great importance in smoke-induced carcinogenesis because it is expected to contribute to the carcinogen metabolism and thus increase the actual concentration of harmful intermediates in the vicinity of target cells. It is also in this type of activation that SO_2 and NO_2 appear to be stimulatory.

The purpose of this article is to summarize some recent work from our laboratory on the stimulatory effect of sulfur dioxide and nitrogen dioxide on the metabolic activation of BP-7,8-dihydrodiol in PML and in more simple *in vitro* systems to reactive DNA- and protein-binding intermediates.

Table 1. Stimulation of tetraol formation by nitrite (1 mM) and sulfite (1 mM) in 12-*O*-tetradecanoylphorbol-13-acetate (TPA) stimulated human polymorphonuclear leukocytes (PML) after 30-min incubation with BP-7,8-dihydroddiol.

Incubation mixture	pmole tetraols/ fold increase
Control	1.0
Control + TPA	1.7
Control + TPA + nitrite	3.8
Control + TPA + sulfite	7.7

The control incubation consisted of 5×10^6 cells and 10 μM BP-7,8-dihydrodiol in a total volume of 3 ml phosphate-buffered saline, pH 7.4.

Results and Discussion

Metabolic Activation of *trans*-7,8-Dihydroxy-7,8-dihydrobenzo[*a*]pyrene

Human PML were incubated with BP-7,8-dihydrodiol in the absence or presence of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (in order to initiate the oxidative burst) for up to 30 min. Aliquots were withdrawn at different time points and assayed for the formation of tetraols (hydrolysis products of BPDE, Figure 2) by high-performance liquid chromatography (HPLC) (*15*). To study the effect of sulfur dioxide and nitrogen dioxide on the metabolism of BP-7,8-dihydrodiol, their reaction products with water, sulfite (SO_3^{2-}) and nitrite (NO_2^-), respectively, were used rather than the pure gases.

The effect of TPA and nitrite or sulfite on the metabolism of BP-7,8-dihydrodiol to tetraols is shown in Table 1. A low basal activity is present in non-TPA-treated cells. Stimulating the oxidative burst by TPA greatly increases the formation of tetraols. Available evidence indicates at least partial involvement of myeloperoxidase (MPO) and H_2O_2 (*16*). The low-basal activity can be explained by the presence of low levels of cytochrome P450 (*17*). Addition of sulfite to TPA-treated but not to nontreated cells greatly stimulates the formation of tetraols. The mechanism underlying the effect probably involves oxidation of sulfite to the sulfur trioxide radical anion ($SO_3^{\cdot-}$), which in turn may react with molecular oxygen to yield a sulfur peroxyl radical ($^{\cdot}OOSO_3^-$). The peroxyl radical may directly or via the corresponding acid epoxidize BP-7,8-dihydrodiol to BPDE (*18*). Like sulfite, nitrite has no stimulatory effect on the tetraol formation in non-TPA-treated cells. However, addition of nitrite to TPA-treated PML markedly stimulates the metabolism of BP-7,8-dihydrodiol. In an attempt to elucidate the mechanism underlying the stimulatory effect of nitrite, extensive *in vitro* experiments have been per-

Table 2. Binding of (^{3}H)-(–)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene metabolites to calf thymus DNA and PMNs proteins.

Incubation mixture	pmole bound/ mg DNA	pmole bound/ mg protein
Control	0.8	7.5
Control + PMA	1.6	21
Control + PMA + nitrite	2.1	45
Control + PMA + sulfite	2.0	9.6

The control reaction consisted of 10 μM (–)*trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene and 10 × 10^6 PMNs in 3 ml PBS, pH 7.4.

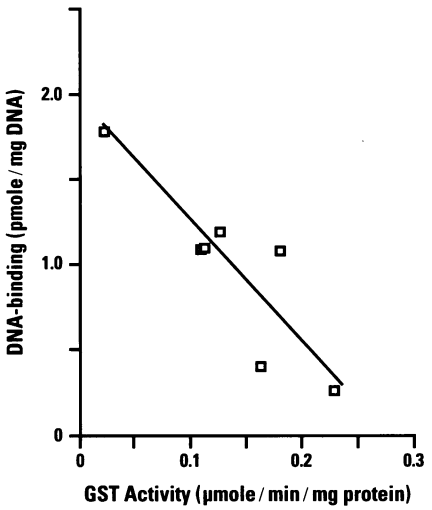

Figure 3. Covalent binding of BP-7,8-dihydrodiol intermediates to exogenous DNA in the presence of polymorphonuclear leukocytes as a function of cytosolic glutathione transferase activity.

formed. In brief, we have incubated purified MPO with its substrate, hydrogen peroxide, and BP-7,8-dihydrodiol in the presence or absence of nitrite. Both tetraols and BPDE could be detected, particularly in the presence of nitrite (~30-fold increase) (unpublished data). Thus we believe that the MPO/H_2O_2 system is the major pathway involved in the nitrite-dependent activation of BP-7,8-dihydrodiol. However, we can not presently exclude significant contribution of additional pathways.

Covalent Binding of Reactive BP-7,8-dihydrodiol Products to DNA and Proteins

The effect of sulfite or nitrite on DNA and protein binding of reactive intermediates from BP-7,8-dihydrodiol has been studied. PML, non-TPA-treated, or TPA-treated, were incubated with BP-7,8-dihydrodiol and exogeneously added calf thymus DNA in the absence or presence of sulfite or nitrite. Binding to exogenous DNA and cellular proteins was estimated as described in (*19*). The results are compiled in Table 2. Initiating the oxidative burst by TPA enhances the binding of BP-7,8-dihydrodiol intermediates to both DNA and proteins. Addition of sulfite or nitrite further stimulates the binding to both DNA and proteins. As evident, the extent of binding to cellular proteins are much higher than binding to DNA. Preliminary experiments involving extensive purification of DNA, enzymatic hydrolysis to nucleosides and subsequent analysis by HPLC indicate that the major DNA-binding intermediate is (+)-*anti*-BPDE, the ultimate carcinogen from BP. Regarding the protein adducts, other intermediates, in addition to those binding to DNA, seem to contribute. For instance, the BP-7,8-dione (probably formed via diol dehydrogenase catalyzed oxidation of BP-7,8-dihydrodiol to the corresponding diol and subsequent nonenzymatic or enzymatic 2 e^--oxidation) is a probable candidate as is the triol epoxide (formed by hydroxylation at the 1 or 3 position of BPDE) (Figure 2). The latter intermediates are highly reactive and exhibits a preference for binding to nucleophilic protein targets (*20*).

Within the concept discussed in this paper, the experiments with DNA localized outside the cells illustrate an important principle, namely that reactive BP-7,8-dihydrodiol products formed within the cells can be released and subsequently react with nucleophilic targets localized outside.

Protection against Reactive BP-7,8-dihydrodiol Products in Polymorphonuclear Leukocytes

Glutathione (GSH) in conjunction with glutathione transferases (GSTs) is the most important cellular system for protection against carcinogenic PAHs. Accepting the hypothesis that PML contribute to the metabolic activation of carcinogens such as BP and thus increase the probability for tumor formation, it is likely that the level of GSH and the qualitative and quantitative distribution of GST isoenzymes are important regulatory factors for the formation and accumulation of reactive BP-7,8-dihydrodiol intermediates. Accordingly, cytosolic fractions from PML isolated from a number of female and male individuals were prepared and assayed for GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) (*21*) (Table 3). In addition, total GST content and isoenzyme distribution were determined by affinity chromatography and HPLC (*22*). The results from this study will be published elsewhere, but it can be concluded that the major GST isoenzyme in PML is GST P1-1, the isoenzyme most efficient in detoxifying BPDE by conjugation with GSH. In addition to GST activities, Table 3 also contains data on total cell protein, protein content of cytosolic fractions and extent of DNA binding of BP-7,8-dihydrodiol intermediates. It is evident that no significant difference exists between females and males with regards to these parameters. Figure 3 shows some results on the relationship between DNA binding as a function of CDNB activities. A negative correlation seems to exist between the extent of DNA binding and GST content. These results may indicate that individuals with high amounts of GSTs are less sensitive to exposure to carcinogens like PAHs than individuals with lower GST activity.

Table 3. Estimation of total protein in polymorphonuclear leukocytes and cytosolic fractions, gluatathione transferase (GST) activity and binding of BP-7,8-dihydrodiol metabolites to DNA.

Source of cells	Cell protein	Cytosolic protein [a]	GST activity [b]	DNA binding [c]
Female	52.4 ± 13.5 (n = 5)	47.4 ± 10.9 (n = 5)	0.14 ± 0.07 (n = 6)	1.05 ± 0.62 (n = 4)
Male	51.5 ± 24.8 (n = 4)	41.2 ± 14.3 (n = 5)	0.13 ± 0.02 (n = 5)	0.89 ± 0.42 (n = 3)

[a] μg protein / 10^6 cells. [b] μmoles 1-chloro-2,4-dinitrobenzene / mg cytosolic protein / min. [c] pmoles BP-7,8-dihydrodiol metabolites / mg DNA.

Conclusions

From our results, it is evident that sulfite and nitrite, major hydrolysis products of sulfur dioxide and nitrogen dioxide, respectively, stimulate the metabolism of BP-7,8-dihydrodiol to both tetraols and to DNA- and protein-binding products in human PML. The metabolic activation of PAHs in PML and the stimulation of this process by sulfite and nitrite may indeed be a contributing factor for the development of primary lung cancer in smokers, particularly in those living in air-contaminated urban areas.

Whereas the mechanism of the sulfite-dependent stimulation is more or less elucidated and involves the formation of a sulfur peroxyl-radical, the mechanism of the nitrite stimulation is still unknown. Preliminary evidence suggests that peroxynitrite, formed through the reaction between hydroperoxide and nitrite, is an important intermediate. How these species interact with myeloperoxidase is, however, not yet evident.

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