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Evaluation of a Possible Role for Antimutagens, Antiteratogens, and Anticarcinogens in Reducing Environmental Health Hazards

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The use of protective agents (e.g., sulfhydryl compounds, certain vitamins, amino acids, cations, and antibiotics) offers a novel and promising means of dealing with the ever increasing burden of environmental hazards facing man. Through the daily uptake of minimal doses as a prophylactic measure by the most endangered groups of the population or by direct mixing of the appropriate protective agent with the inducer (e.g., pesticides or anticancer drugs) it should be possible to reduce or prevent some of the most serious toxic side effects including those of a mutagenic, teratogenic or carcinogenic nature. Among some of the most outstanding protection examples cited are the antimutagenic, antiteratogenic and anticarcinogenic effects of *L*-cystein, and of some of the vitamins. However, in view of our limited understanding of protection mechanisms in this fairly new field of research and due to the toxic side effects of some of the protection agents themselves, a large-scale application of this approach cannot be recommended as yet. More research is urgently needed to study protection mechanisms in suitable standardized model systems and to develop safer and more efficient protective agents.

Introduction

Now that the negative consequences of industrialization on the environment have gained general recognition, more attention should be devoted to finding practical ways to minimize effects on present and future generations.

In considering those environmental hazards which have no useful function, e.g., car exhaust, or those which can be replaced by less toxic substitutes, e.g., thalidomide, the necessity for their elimination should be too obvious for discussion. The picture is different, however, when we consider agents having a vital role and which are not readily replaceable e.g., pesticides and anticancer drugs. Until less toxic substitutes are found, an effort should be made to reduce their undesirable side effects on man. One way to attain this objective could probably be achieved through the use of protective agents, i.e., certain compounds such as those with SH groups, some vitamins, and amino acids which, upon application in conjunction with certain toxic agents, reduce or inhibit their toxic effects.

Rather than to attempt to cover the whole field, which has been subject to some excellent recent reviews on antimutagens (*1*) and anticarcinogens (*2,3,*), the present evaluation will be oriented mainly to (a) emphasize the universal aspect of the protection phenomenon across several types of toxic effects and different biological systems, (b) discuss some of the protection mechanisms, and (c) evaluate the prospects of using these agents to cope with some of today's environmental hazards.

Our present understanding of the mechanisms involved in the protection activity has not yet reached the stage which would allow us to recommend the practical use of protective agents at the present time. This report should, however, serve as a stimulus for the search for nontoxic protective agents as well as to intensify the study of structure activity relationships with a view to a better understanding of the mechanisms involved.

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The Universal Aspect of the "Protection" Phenomenon

There is at present sufficient evidence to prove that certain chemical agents, including SH compounds, amino acids, vitamins, nucleic acid precursors, cations, and antibiotics, are capable of showing antimutagenic, antiteratogenic and/or anticarcinogenic effects if applied in conjunction with the respective toxic agents. These "protection" effects are not to be confused with therapy, since both protector and inducer are applied in these experiments either simultaneously or within a short time of each other.

The protection phenomenon has some unusual and fascinating aspects. First, the grave consequences of mutagenesis, teratogenesis, and carcinogenesis, normally regarded as irrevocable, could be inhibited by the mere application of a protective agent. Second, protection by a given agent is not specific but is, at least in most cases where sufficient data are available, of a universal nature; a protector being capable of acting against several types of inducer in a variety of biological systems ranging from microorganisms to mammals. It would certainly be advantageous to develop model systems, both *in vitro* and *in vivo*, which would permit us to gain a better insight into this universal aspect of the "anti" activity. The following examples should serve to demonstrate this universal action of protective agents.

SH Compounds

As a representative of this group, we shall consider L-cystein, one of the best known protective agents. L-Cystein was shown to protect human cultured leukocytes against the clastogenic effects of 2,3,5 trisethyleniminobenzoquinone-1,4 (Trenimon) and busulfan (*4*), both being alkylating agents, as well as against 8-oxyquinoline sulfate (*5*), a nonalkylating clastogen. Besides this antimutagenic effect, L-cystein was also found to inhibit the teratogenic effects of tetramethylthiuram disulfide (Thiram) (*6*) as well as the carcinogenic effect of dibenzanthracene (*7*).

Vitamins

The anticarcinogenic effects against polycyclic hydrocarbons of vitamin A, has been known for several years (*2,8*). A 0.5% vitamin A palmitate diet protected against the effects of 7,12-dimethylbenz[a]-anthracene (DMBA) (*9*) in the induction of cancer of the forestomach and small intestines as well as dyskeratotic lesions of the esophagus in Syrian hamsters. In other experiments, 5 mg vitamin A per stomach tube prevented the appearance of squamous metaplasia induced by an intratrachial application of benzo a pyrene (BP) in Syrian hamsters (*10*).

A third example of the anticarcinogenic action of vitamin A could be seen in protection of rhino mouse skin treated with DMBA after supplementation of the diet with vitamin A (*11*).

Riboflavin, another protective vitamin, was also found to inhibit liver carcinogenesis in rats treated with 4-dimethylamino-azobenzene (DAB) at a level of 200 μg riboflavin per rat in a diet containing casein (*12*). In addition to its anticarcinogenicity, riboflavin protected against the teratogenic effects of both hypoglycine A (*13*) and boric acid (*14*).

Vitamin C made prominent news when it was recently found (*15*) to prevent the formation of nitrosamines from amines and nitrites *in vitro*. This finding was later confirmed *in vivo*, a protective effect of vitamin C having been observed in rats treated with ethylurea plus sodium nitrite. By preventing the formation of nitrosoethylurea, vitamin C protected the treated rats against both the teratogenic (*16*) and transplacental carcinogenic effects of nitrosoethylurea (*17*). Since nitrosamines can be formed in nitrite-treated food (*18*) and could also occur in certain pharmaceutical drugs (*19*) and because of the mutagenic, teratogenic, and carcinogenic potential of nitrosamines (*20*), there is a good possibility that vitamin C be considered for use as a protective agent in endangered foods and drugs.

Amino acids

So far, only a few members of this group have been shown to have protective effects. L-Glutamic acid (but not the D-isomer) was reported to protect against *N*-phthalyl-DL-glutamic acid, a teratogenic metabolite of thalidomide (*21*). The observed effect could not, according to these authors, be simply due to a compensation for the known glutamic-antagonistic action of the teratogen. L-Asparagine and, to a lesser extent, L-methionine showed an anticlastogenic effect against Trenimon in human lymphocyte cultures (*22*).

Nucleic Acid Precursors

All four nucleotides TdR, AdR, CdR, and GdR showed protective effects against lethal mutations

induced in *Drosophila* by x-rays (*23*). TdR showed, in addition, an antiteratogenic effect against hydroxyurea in rats (*24*).

Cations

Among the cations showing protective effects, zinc was found to have both antiteratogenic as well as anti-carcinogenic potential. Zinc carbonate protected against EDTA-induced teratogenicity (*25*), while zinc acetate proved efficient in countering the carcinogenic effects of cadmium chloride on the testes of mice and rats (*2*).

Sodium cobaltinitrite was also found to protect mice against skin carcinogenesis induced with methylcholanthrene (MC) (*26*).

Trace amounts of cupric oxyacetate in the diet protected against cancer of the liver (but not that of the skin and ear duct) after treatment with 3-methoxy-4-aminoazobenzene (*27*).

Another cation, vanadium, applied in the form of vanadium pentoxide to the skin of mice treated with MC prevented the appearance of induced tumors (*28*). Finally, Na-fluoride proved to be antimutagenic both in cultured human leukocytes (*29*) and in drosophila (*30*); the cultured cells were protected against the clastogenic effects of three polyfunctional ethylimines, whereas Drosophila was protected against mutagenesis by Trenimon and 1-phenyl-3,3-dimethyltriazine.

Antibiotics

Both chloramphenicol and actinomycin D were found to have protective effects. Chloramphenicol protected *Drosophila* against mutagenesis by x-rays (*31*) and showed an anticlastogenic effect against both cyclophosphamide in HeLa cells (*32*) and rubidomycin in bone-marrow cells (*33*). Its activity against carcinogens was also apparent in protecting rats against cancer induction by the azo dyes: *N*-2-fluorenyldiacetamide (*34, 35*), DAB (*36*), and 3′-methyl DAB (*37*).

Actinomycin D showed an antimutagenic effect both in yeast (*38*) and *Drosophila* (*39*) as well as an anticarcinogenic effect against several carcinogenic hydrocarbons (*3*).

The above examples, by no means exhaustive, serve to show that protection is not a specific phenomenon. The same protective agent is often capable of countering the damaging effects of several types of toxic agents in different systems. This universality of the protective effect, however, does not apply to the mechanism of protection. A multiplicity of mechanisms must be assumed to explain the protection effects induced by such agents as cations, nucleic acid precursors and antibiotics, to mention a few. Even if we consider the protection mechanism of a single agent, L-cystein, for example, we have to conclude that more than one single mechanism must be at play which would explain the action of L-cystein against such inducers as radiation, alkylating and nonalkylating chemicals.

Our present knowledge of the multiplicity of mechanisms taking part in protection is unfortunately very limited. It would, nevertheless, seem profitable for our present discussion to point out some of the probable mechanisms playing a part in the protection effects observed in the examples cited above.

Mechanism of Protection

It should be possible theoretically, once the mechanism of action of both inducers and protectors are known, to be able to plan protection against a given inducer by selecting the optimal protector and conditions most suited to counter its action. With our present state of knowledge, however, we are far from achieving this goal. Success in protection experiments is usually achieved more by trial and error than by design. The reason for this is the multiplicity of factors playing a role in the protection phenomenon and which render a full understanding of the process very difficult to attain. Among these are: the dose-ratio of protector to inducer; the sequence of application of both agents; the time lapse between the application of both agents, the route of application of each agent, the biological system used, the kinetics of absorption and activity of each agent, etc. The time lapse between the application of inducer and protector is usually very short in protection experiments against mutagens and teratogens but could extend over a whole month (the initiative stage) in experiments dealing with carcinogens (*2*).

There are many possible mechanisms of protection, including, among others, a direct reaction between the two agents, an induced decrease in drug metabolizing activity, competition for binding sites by the protecting agent, competitive inhibition, and a stimulation of drug-metabolizing enzymes. However, stimulation of these enzymes could lead to protection only if the resultant metabolites are less active than the intact inducer itself (*3*). If, on the other hand, metabolites with a higher activity are produced through the action of microsomal enzymes, as is the case with nitrosamines, then an enhanced toxic activity rather than protection will result (*40*). Several agents are known to induce

microsomal enzymes. Among these are several polycyclic aromatic hydrocarbons; DDT, dieldrin, barbiturates and others (*41*). The protective role of induced enzymes in carcinogenesis has been reviewed (*17, 42*). In considering the protective action of barbiturates, as one example of an enzyme inducer, we find that whereas a stimulation of hydroxylases seem to explain the protective role of amylobarbitone against Chlorambucil and Melphalan (*43*), a different, and so far an unknown mechanism, appear to be involved in the protective effect of phenobarbital against nephrotoxicity induced by methylmercury (*44*).

Protection could also be mediated by a stimulation of repair processes, especially during the labile mutation fixation period (*45*). If the protector itself is mutagenic e.g., chloramphenicol (*46*) and actinomycin D (*47*), then we could expect that part of the observed protection effect be due to genetic alterations favoring the appearance of the wild type e.g., back-mutations, and recombination processes.

In the case of anticarcinogens, we could expect, in addition to the above, that tumor incidence be reducible through a cytostatic or a cytocidal effect by the protector on the initiated cells. Another possibility for protection against the formation of tumors lies in a stimulation of the immune system by the protector to act against tumor formation. Antiteratogens could also indirectly act by countering nonspecific toxic effects of the inducer on the uterus.

In view of this multiplicity of possible mechanims, some of which might be operating simultaneously either synergistically or antagonistically, any attempt at oversimplification in analyzing the mechanisms involved in a given protection experiment will be dangerous. It is with this precaution in mind, that the following discussion on mechanisms should be considered.

Sulfhydryl Compounds

These "universal" protectors are thought to act against radiation effects mainly by a scavenging of radicals (*48,49*). Their action against the multiple chemical agents listed above might involve the stabilization of enzymes through the formation of enzyme–S–S–protector complexes (*50*), participation in repair processes. (*51*), and others. The exact mechanism in each case is unknown, however.

Vitamins

Besides their known role as coenzymes in certain metabolic reactions, the protective effects observed after application of excess amounts suggest other functions of vitamins applied in conjunction with the different inducers. A workshop sponsored by the National Cancer Institute and Hoffmann-La Roche, Inc. was held recently in Bethesda on the anticarcinogenic action of vitamin A (*52*). The impression gained from the evidence presented by the participants seems to suggest that a chronic vitamin A deficiency is associated with an increase in potency of several carcinogens. The protective action of added vitamin A was interpreted in one report as an inhibition of benzo[a]pyrene (BP) binding to DNA and in another as an inhibition of BP oxidation (activation) to its presumed carcinogenic form. However, the anticarcinogenic effect of vitamin A was not limited to carcinogens needing activation. Vitamin A, a known adjuvant, is also thought to act against carcinogenesis by stimulating the immune system. Retinyl palmitate was reported to potentiate by a hundredfold the antitumor effect of BCG.

Riboflavin is thought to antagonize the inhibitory effect of hypoglycin A directed against the acyldehydrogenase flavine-dependent oxidation reaction (*13*). Riboflavin-supplementation protects against azo-dye carcinogenesis, probably by replenishing the dangerously lowered endogenous riboflavin level observed in the liver of rats treated with DAB (*53*). This vitamin seems also to play a role in the activation of microsomal enzymes including azo reductases (*54*).

The protection of vitamin C against the formation of nitrosamines from their components could probably be explained by competition of this vitamin with nitrous acid for combination with the amines; at least this appeared to be the mechanism operating *in vitro* (*15*). The antiteratogenic effect of vitamin C against 3-acetylpyrimidine and 6-aminonictinamide (*55*) is thought to be mediated through a stabilization of mitochondrial structures attacked by the teratogen.

Amino Acids

L-Asparagine is postulated (*22*) to protect human lymphocytes from the clastogenic effect of Trenimon by stimulating the synthesis of nucleic acid precursors.

Nucleic Acid Precursors

It can be assumed that, by helping re-establish DNA and RNA synthesis in cells where this synthesis is inhibited by the inducer, these precursors could thus offset some of the damage induced (*24*).

Cations

The role of various cations in protection can only be guessed. One possibility is that the small applied protection dose acts as a catalyst to speed the breakdown of the inducer to less active components (*8*).

Antibiotics

In the case of protection against *N*-2-fluorenyldiacetamide, chloramphenicol is thought to act by competing with the carcinogen for certain enzymatic active sites (*35*). Some possible explanations of actinomycin D protection mechanisms, are given in a detailed discussion by Van Duuren and Melchionne (*3*).

Protective Agents as a Possible Tool to Reduce Environmental Hazards

Even with our present limited knowledge of mechanisms, there are situations where the use of protective agents could eventually be envisaged as a possible means for reducing environmental hazards. In the following we shall cite some examples.

As a Prophylactic Measure

Dietary supplements of appropriate protective agents could possibly protect people working with genetically active agents. Among these are workers in oil refineries, in the manufacture of asbestos, certain pesticides, PVC, and alkylating agents. Evidence for the carcinogenicity of the last group of chemicals in man has just been published (*56,57*).

As a Preventive Measure against Nitrosamines in Food

The use of vitamin C in this respect is only justified if its presence does not also protect bacteria (especially those of the botulinus type) against the antibiotic effect of nitrite in food. Botulism is obviously more dangerous than the presence of traces of nitrosamines.

As a Protector against Side Effects of Anticancer Therapy

Reducdyn (a combination of *N*-acetylhemocystein-thilactone, L-cystein, and fructose) is already being successfully used against the adverse effects of irradiation (*1*) and of Trenimon (*58*). Recently, thymidine (TdR) was shown to protect against the toxicity of methotrexate while maintaining its antitumor effects in mice bearing leukemia L 1210 (*59*). Cysteamine, as well as β-aminoethyisothiouronium (AET) showed promise in reducing the clastogenic effects of the anticancer drug Trenimon without affecting its cytostatic action in cultured lymphocytes (*60*). WIth further research, it should be possible to reduce the toxic side effects of other agents used in cancer therapy.

As Protection against the Side Effects of Antibiotics

Most antibiotics have undesirable side effects, some of a genetic nature. For example chloramphenicol is mutagenic in yeast (*46*) and teratogenic in the rat (*61*). Actinomycin D is mutagenic in the mouse (*47*) and teratogenic in Syrian hamsters (*62*). Gentamicin, mitomycin, streptonigrin, patulin, phleomycin, and daunomycin induce chromosome abberrations in cultured cells (*63*). It is therefore certainly worth the effort to find protective agents which would reduce these side effects without much interference with the desirable antibiotic action.

As Protection against Side Effects of Hormones

Any success achieved in this field will be beneficial not only to patients undergoing hormonal therapy (cortisone is a known teratogen; estrogen is a suspected carcinogen) but to the millions of women using oral contraceptives, drugs now suspected of being associated with the appearance of some hepatomas (*64, 65*).

As Protection against Other Environmental Hazards

Some promising results show that protection can be achieved even against air pollution by a dietary supplement of vitamin E. This was shown in rats treated with simulated smog (*6*). Vitamin A was also cited above as a protector against several carcinogens in rats (*10*). The risks and benefits to man of these protective agents should be thoroughly investigated.

In view of the promising aspects of these results and their possible future use in solving some of today's environmental problems, it is hoped that environmental agencies encourage more research in

this field with a view to a better understanding of the mechanisms and the development of effective protective agents against the environmental hazards mentioned.

It must be understood, however, that protective agents known at present offer no panacea, since many of them have their own undesirable side effects. the teratogenic and mutagenic effects of chloramphenicol and actinomycin D have already been mentioned. Vitamin A, at doses higher than the daily requirement has long been known to be both toxic and teratogenic (*66, 67*). L-Asparagine at high doses was found to induce brain lesions in mice (*68*). NaF increased γ-irradiation-induced recessive lethality in *Drosophila* (*69*). A similar effect in bacteria was observed with zinc chloride (*70*). Even L-cystein was capable of increasing rather than decreasing Thiram-induced lethality in rats (*71*). There are also several anticarcinogens which are themselves carcinogens (*3*).

Nevertheless, rather than be discouraged by these difficulties, the promising benefits to man should encourage us to intensify our efforts to gain a better understanding of protection mechanisms by using model systems which permit a systematic study of structure–activity relationships and to find better protective agents with less toxic side effects.

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