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Methodological Aspects of Studies of Chemical Mutagenesis Modification

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Based on analysis of mainly own data the author discusses the methodological problems of studies of modification of chemical mutagenesis, offers general recommendations for planning and realization of experiments, and analyzes possible trends in research and prospects for practical application of the results.

Key Words: *mutagenesis; antimutagenesis; comutagenesis; drugs; nutrients*

Prevention of delayed effects of induced mutagenesis is based on screening of mutagens and prevention of their contacts with humans. Systems of various degree of complexity for mutagen screening of drugs, cosmetic and nutrient substances, and other chemicals are methodologically validated, developed, and are widely used. Regulations and experimental protocol of testing for mutagenicity and problems in interpretation of the results are fixed in a series of research reports and normative documents [12,13,32,35,37]. However, despite all efforts, an appreciable part of the population is regularly exposed to mutagens of different nature [34].

Studies of mutagenesis modification are a perspective trend of practical prevention of mutagenesis [4,14,30]. Methodological and technological approaches to studies in this sphere greatly vary [42]. This impedes the analysis of the results and evaluation of their significance, inhibits introduction of antimutagens as preventive means, and development of measures for prevention of comutagenic exposure.

Pharmacological approach, which was initially validated at the beginning of the 20th century [15, 27], serves as the basis for the search of methodologies for investigation of mutagenesis modification. We analyze here general problems of studies of mutagenesis modification and sum up the results of studies in this sphere, carried out at Institute of Pharmacology.

Induction of mutations is a multistage process consisting of the entry, distribution, biotransformation, and elimination of the genotoxicant, its accumulation in the cell and target molecule, interactions with DNA, and activities of the reparation and other defense systems [8,15]. It is obvious that modification of any of these stages of mutation development modifies the mutagen effect. This assumption is illustrated by numerous classical examples of modification of the effects of indirect mutagens. Their effects are attenuated or stimulated depending on the inhibitors or inductors of cytochrome P-450 system enzymes.

Many mutagens realize their damaging effects by inducing oxidative stress and the formation of endogenous mutagens (free radicals, peroxides, aldehydes) associated with it. In this case mutagenesis can be modified by modulation of antioxidant defense efficiency, which is confirmed by data on the mutagen-modifying activity of exogenous antioxidants [7,15]. Reparative activity of some antimutagenic compounds was reported [15].

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Hence, each component of mutation development can be regarded as a pharmacological target for mutagenesis modification, and hence, we can abandon the empirical search and concentrate on studies of mutagenesis modifiers.

Importantly that the same compound can modulate not just one, but several targets at once and modify the mutagenic effects in different directions, in some cases exhibiting antimutagenic, and in others comutagenic effects. The latter is typical of many natural and synthetic antioxidants, particularly phenol derivatives [7,15].

Classification of mutagenesis modifiers

Two circumstances impede the classification of mutagenesis modifiers: polypotency of the modifiers effects and a wide spectrum of biosystems (from microorganisms to human cells), used *in vivo* and *in vitro* in studies of mutagenesis modification. It is virtually impossible to create a clear-cut qualification system, despite bright solutions proposed by M. D. Waters *et al.* [41], and therefore the known summations are merely formal.

We shall use here only two unquestionable classification terms, reflecting opposite directions of mutagenesis modifications: antimutagen and comutagen (substances attenuating and stimulating, respectively, the genotoxic effects of mutagens).

A principally important fact is that the compounds with mutagenic potential cannot be regarded as mutagenesis modifiers. All variants of their interactions with other mutagens can be adequately described within the framework of pharmacological notions on the synergic, additive, or antagonistic effects of biologically active substances. We formulated this assumption previously [15] and many well-known authors share this viewpoint [42].

Methodological aspects of studies of mutagenesis modification

Proceeding from the principle of a pharmacological target in studies of the phenomenon and mechanisms of mutagenesis modification by chemicals, we can say with good grounds that mammals are the most adequate test system for these studies. The use of these animals offers a unique possibility to detecting the effect realized by any known or hypothetical mechanism, study the dose-effect relationships, protocol and route of administration, and obtain the data of prognostic significance. This does not preclude the use of other test systems for primary screening of modifiers or analysis of special problems. By contrast, the use of *in vitro* test systems or prokaryotic test systems provides incomplete data, which have to be verified on animals in any case. It is principally impossible to detect the effects of antimutagens, realizing their protective action through second messengers, to study the pharmacokinetic parameters, formation of endogenous interferon and other endogenous antimutagens (ubiquinone, glutathione, antioxidant defense enzymes, *etc.*) in experiments on microorganisms.

Therefore, we shall discuss here the problems connected exclusively with experiments on mammals.

Genotoxicological experiments on mammals are carried out primarily for studies of chromosome aberration induction (methods for estimation of chromosome aberrations and/or micronuclei) in a limited number of tissues [15,33,37].

It may seem that the impossibility of modulating *in vivo* the induction of gene mutations is a great drawback. However, experience gained in genotoxicology suggests that there are no mutagens inducing specifically gene or chromosome mutations alone. Hence, we can suggest that the modifying effect is also universal, involving both categories of mutations, as it modulates the common mechanism of their induction.

The problem of tissue specificity of the effects can be in future solved by the DNA comet method used for evaluation of the pre-mutation events virtually in any mammalian tissue *in vivo* [10,36]. However, at present the problem on quantitative proportions of DNA damage and various categories of mutations remains unsolved.

Choice of mutagenesis inductors

The majority of authors choose mutagens for experiments on mutagenesis modification on the basis of preceding experience. In Russia, dioxidine is used most often, while in foreign countries cyclophosphamide is preferred. We worked with dioxidine and cyclophosphamide in our experiments. Dioxidine is a prooxidant mutagen virtually not metabolized in the body; cyclophosphamide is an indirect alkylating agent with free radical component of action. Comparison of the data obtained in parallel experiments with these agents permits cross-verification of the data and allows the researchers to put forward well-based hypotheses on the mechanism of the observed modification.

It is not the choice of a certain mutagen, but the mechanism of mutagenic effect realization that is important.

The choice of the level of modification is also essential for obtaining reliable results. The probabi-

lity of false results drastically increases in experiments with low or extremely high levels of damage. The optimal level of modification in studies by the method of chromosome aberrations estimation is 10-12%. This is confirmed by our results and published data.

Choice of methods and protocols of substances administration

Basic pharmacological notions indicate that biological effect of the substance depends greatly on the route and protocol of administration. The choice of these constituents should be determined by the tasks of the experiment.

The mutagens are injected parenterally, while expected modifies are administered orally in the majority of the known phenomenological studies. This prevents direct reactions between the substances or their mutual effects at the stage of gastrointestinal absorption, which excludes these possible mechanisms of modification (by which, for example, the protective effects of nutrient fibers are realized [23]) from these studies. However, absorption characteristics of the modifier or its high reaction capacity towards the potential mutagen are usually known beforehand and therefore, the routine approaches can be corrected for the experiment.

A more complex problem is the protocol of administration. For example, aspartame (food sweetener) exhibits antimutagenic activity after a single, but not after 5-fold co-administration with dioxidine and cyclophosphamide. By contrast, β-carotene stains are inert after single dose, but reduce the effects of these mutagens under conditions of repeated co-administrations [21,25,29]. In addition, the modifying effect depends on the time of substance administration in relation to the mutagen. For example, ubiquinone-10 exhibits antimutagenic effects only when used 24 and 1 h before and 1 h after dioxidine injection and is ineffective if administered during the intermediate period [6].

The traditional virtually simultaneous single administration of the mutagen+modifier pair does not fully demonstrate the modifying potential of the studied substance. The data of pharmacokinetic studies are helpful in optimization of the study, but they are as a rule carried out on substances with known activity. Palliative solution can be found by using modified special protocols of the study (Table 1). This approach, used at Institute of Pharmacology, was tried with good results not once and provides characterization of the modifying potential of the substance realized by all mechanisms, including those with delayed effects.

Reliability of results

Evaluation of the reliability of the results of experiments on mutagenesis modification should be based on statistical conclusions about differences between the groups, analysis of dose-effect relationship, and reproducibility of the observed effect upon different protocols of administration. This approach improves the reliability of the final conclusion and helps to detect the compounds liable to inversion of antimutagenic and comutagenic effects.

The numbers of damaged cells and special categories of DNA and/or chromosome damage should be compared, because this provides additional often very important information on regularities of the mutation process modification by a certain substance [4,28,39].

MAIN RESULTS AND TRENDS OF RESEARCH DEVELOPMENT

Comutagenesis

The need in comutagen screening has been validated previously; data on drugs and nutrient comutagens [14] were confirmed and supplemented in later studies. For example, comutagenic activity of calcium channel blockers, previously revealed *in vivo* [38], was confirmed in experiments with ribavirin [9]. High sensitivity of blood cells to some mutagens was revealed in donors treated with vitamin complexes of certain quantitative and qualitative composition [40].

TABLE 1. Protocol of Cytogenetic Experiment for Evaluation of Induced Mutagenesis on Mice Used at Institute of Pharmacology, Russian Academy of Medical Sciences

Administration	Presumable modifier (orally), 3 doses	Mutagen (intraperitoneally), 1-2 doses
Single	Once, for 6 and/or 24 h	Once, for 6 and/or 24 h
Preliminary	Daily for 5 days	Once, together with the last dose of modifier*
Combined	Daily for 5 days	Daily for 5 days

***Note.** *Animals were sacrificed 6 and/or 24 h after administration.

Comutagens possess no mutagenic activity of their own, and therefore they easily pass through the genetic screening "sieve", but can play an important role in increase of genetic burden of the population. Comutagenesis is virtually a terra incognita, and studies in this sphere are an important trend with the main aim of creating systems of comutagen screening.

Antimutagenesis

Antimutagenic modification of mutagenesis is regarded as the biological base for protection of human heredity. Development of studies in this sphere survived a crisis associated with conjunction of antimutagenic and comutagenic properties, characteristic of some phenol antimutagens/antioxidants and apprehensions of dose-dependent and/or tissue-specific inversion of the antimutagenic into comutagenic effect [15].

Modern state of the art is characterized by detection of antimutagens not liable to inversion of the effect, while the use of the DNA comet method [10,36] and polyorgan micronucleus test [26] is expected to clear out the problems of tissue-specific effect of antimutagens.

In general, research of antimutagenesis develops in two directions.

One of them is pharmacological trend, consisting in the search for approaches to arresting the effects of compounds (primarily drugs) with *a priori* mutagenic activity, which however have to be used for medical purposes, and control of abnormally high level of mutations observed in some diseases and in individuals with harmful habits (Table 2).

These studies are based on detailed investigation of the mechanisms of mutagenic effects and suggest the creation of specific pharmacological correctors of mutagenesis [7,8].

By its tasks, the development of a mutagenesis corrector virtually does not differ from the development of any drug based on a synthetic or natural compound. The presumable corrector is not only to possess high specific antimutagenic activity towards selected mutagen or a group of mutagens with similar mechanisms of damage, but is also to meet all requirements to drug safety. Properly constructed pharmacological antimutagen can completely eliminate the mutagen effect, which is confirmed by the results of experimental and clinical studies [7,15].

Antimutagenic effects of some drugs and candidate drugs were detected and comprehensively studied by the pharmacological method. These are 1,4-benzodiazepine, 2-mercaptobenzimidazole, 3-hydroxypyridine derivatives, crown compounds, and some others [7,15,19]. It is noteworthy that 2-mercaptobenzimidasole derivatives exhibited antimutagenic properties not only in experiments with chemical mutagens, but also in parasitic invasions [3]. A new derivative of this group, afobazole (anxiolytic, created in Russia), seems to be a promising pharmacological antimutagen; this agent exhibited protective effects in a wide range of doses [17] and in different tissues towards many chemical mutagens (author's unpublished data).

TABLE 2. Diseases and Conditions Associated with Oxidative Stress, Increased Incidence of Chromosome and/or DNA Aberrations (Author's and Published Data)

Diseases	Conditions
Fanconi's anemia	Tobacco smoking
Bloom's syndrome	Mental stress
Ataxia-telangiectasia	Intoxications of different origin
Diabetes	Avitaminosis and vitamin deficiencies
Asthma	Aging
Hepatitis	
Schizophrenia	
Alzheimer's disease	
Parkinson's disease	
Autoimmune diseases	
Helminthiasis	
Viral infections	
Bacterial infections	
Malignant tumors	
Atherosclerosis	

The idea of creating pharmacological correctors of mutagenesis was in fact realized in collaboration with S. B. Seredenin, Member of Russian Academy of Medical Sciences. Clinical studies demonstrated positive results of using bemethyl (actoprotector) for prevention of mutagenic effects of dioxidine (a valuable antibacterial agent) and rutin (flavonoid) in Fanconi's anemia [15] and of dimephosphone (phosphonic acid derivative) in atopic bronchial asthma (Fig. 1) for reduction of abnormally high level of chromosome aberrations in blood cells of these patients.

The other trend, nutriciological, consists in the search for approaches to increase of resistance to mutagenic exposure caused by nutrients or foodstuffs. The most demonstrative example of studies in this direction is evaluation of the role of essential nutrients in the formation of resistance to mutagenic exposure. For example, our studies showed that some (but not all) vitamin complexes of certain quantitative and qualitative composition can appre-

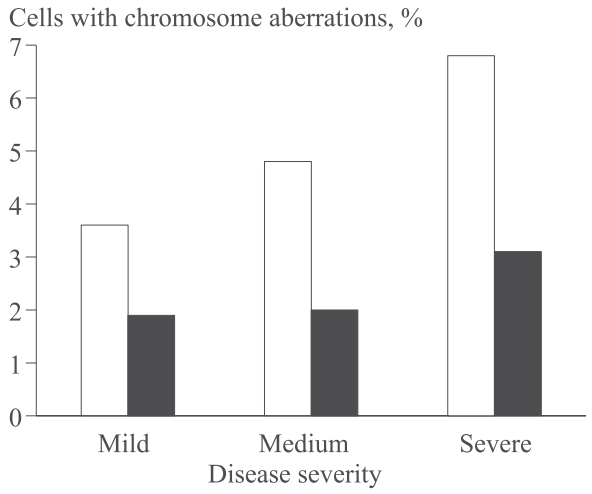

Fig. 1. Cytogenetic studies in patients with asthma of different severity receiving therapy including dimephosphone (according to A. V. Semenov data). Light bars: before; dark bars: after therapy.

ciably improve human cell resistance to cytogenetic exposure [16,40].

Studies aimed at creation of antimutagenic functional foodstuffs and bioactive additives within the framework of this trend of research are of special importance [11]. The development of these studies is based on extensive screening of antimutagenic properties of nutrient compounds. Our studies on mammals detected antimutagenic effects of carotenoid and anthocyan dyes [2,22,24], aspartame (sweetener) [21], natural metabolites; ubiquinone [6], betain [20], lipidovite [1], oak wood extract [5], birch bark extract [18], baikolinates, *etc.* [15,27]. Antimutagenic functional products and bioactive additives were created on the basis of these agents and tried with good results [23,25]. On the other hand, the efficiency of these compounds and products created on their basis is low, and they reduce the mutagenic effect in experimental studies by no more than 25-40%. The only exclusion is dry extract of birch bark (DEB) containing up to 70% betulin [18]. Its protective effect is comparable to that of pharmacologically active derivatives of 2-mercaptobenzimidazole, which (according to analysis of published reports) can be regarded as the most effective of known antimutagenic compounds. Clinical confirmation of DEB efficiency will open wide prospects for its multifunctional (drug, bioactive or food additive) use for the protection of human genome.

Many known antimutagens belong to plant substances, primarily foodstuffs. In addition, some synthetic food components, for example, aspartame (sweetener) exhibited antimutagenic effects [21,24]. Together with the known ideas about alimentary chemoprophylaxis [30,31] and functional foodstuffs [11], these data can give rise to ungrounded optimism in food antimutagenic prophylaxis. We should like to emphasize in this connection that just separate compounds, isolated from foodstuffs, were analyzed, but there is in fact no information about antimutagenic activity of foodstuffs containing alimentary antimutagens. Our experience indicates that the desired effect can be rarely attained by just adding the alimentary antimutagens to food and/or their preservation in ready products. The antimutagenic effect of individual alimentary compounds in the alimentary biological mass is leveled in the majority of cases. The only exclusion are alcohol-free drinks; we previously described the philosophy of creation of antimutagen products on this basis [11,24].

CONCLUSION

The problem of genome protection by pharmacological and nutritional antimutagens is far from solution, despite the obvious good prospects of development and the first positive clinical experience of their practical use. The effects of the overwhelming majority of the known antimutagens on germ cells are not disclosed, the problem of possible comutagenic effects is not cleared out, particularly for "secondary" tissues, and approaches to evaluation of the efficiency of antimutagenic prevention are not developed. In addition, formal problems of prescription of pharmacological antimutagens and use of antimutagenic foodstuffs for prevention of probable and delayed effects of mutagenesis remain unclear. On the other hand, antimutagenic prophylaxis and prevention of contacts of humans with mutagenic and comutagenic substances will help actually reduce the mutagenic exposure of humans. The use of pharmacological approaches and methodology in mutagenesis modification studies will appreciably facilitate the search study, and introduction of antimutagens in preventive medicine.

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