REVIEWS

Co-Mutagenesis as New Vistas in Genotoxicology

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The leading role of induced mutagenesis in the maintenance of the level of hereditary, oncological, and some other pathological conditions is widely known. The main groups of mutagens were described and approaches and methods of their rapid detection in the environment were developed [7,10].

At the same time, the problem of co-mutagenesis, *i.e.* potentiation of the damaging effect of mutagens by non-mutagenic agents, received less attention. Co-mutagens have no intrinsic mutagen activity and are not detected during genotoxicological screening. Uncontrolled presence of co-mutagens in the environment can potentiate the negative effects of industrial, medicinal, and other mutagens, which are necessarily come into contact with humans [12].

In this paper we summarize the data of our investigation in the field of chemical co-mutagenesis, analyze published data, and formulate new actual problems of co-mutagen detection.

History

The phenomenon of co-mutagenesis was first established in the experiments on microorganisms during the study of harman and norharman, heterocyclic amines produced from tryptophan during thermal treatment of dietary proteins [21,35].The co-mutagen properties of these agents were demonstrated in experiments on cultured eukaryotic cells, but not in mammals *in vivo* [31].

Further experiments demonstrated co-mutagenic activity of some natural and synthetic organic and inorganic compounds. However, until present these data are very scarce and are mainly focused on the description of the results obtained in elementary microbiological test systems [21]. The prognostic value of these results is limited due to problems of their extrapolation to humans. For the same reason, the data obtained on eukaryotic cells *in vitro* are also not conclusive [5,12,19]. Therefore, in this paper we consider experimental data obtained *in vivo*.

Paucity of the data on *in vivo* co-mutagenic effects can be explained by the fact that routine genotoxicologic research are aimed at detection of genotoxicologic, but not mutagen-modifying activity of the examined compounds. Moreover, there are no special programs focused on detection of co-mutagens. The experimental studies aimed at the detection of co-mutagenic properties are virtually absent. Most data about co-mutagens were obtained as a side results during the search for and the study of antimutagens.

Methodical Peculiarities of Co-Mutagen Studies

For evaluation and description of potential mutagenic activity investigators use various genotoxicity test systems. These tests detecting various categories of gene aberrations are performed *in vitro* on pro- and eukaryotes and/or *in vivo* on intact organisms of different evolutionary levels [7,10]. The same tests can be used for analyzing mutagen-modifying, and first of all, antimutagen activity. However, in this case *in vivo* tests, *e.g.* the count of chromosome aberrations in the bone marrow cells in mammals, are more preferable. These tests identify the majority of known and hypothetical mechanisms of mutagenesis modification [2].

The objective of these tests is detection of various modifications of the effects of known mutagens. The use of standard mutagens raises some methodical problems. First of all, this is the choice of "standard" mutagens, the order and time of administration of mutagen

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and modifier, mode and regimen of administration, doses of the examined agents *etc*. The guidelines for this multiple choice could be elaborated from the data on pharmacokinetics of the test substances. However, in most cases (for example, for plant extracts) there are no such data. Therefore, the design of the experiment is developed according to personal experience. On the basis of our previous experimental results [12] we propose the design of *in vivo* experiment with peroral administration of the test modifier and intraperitoneal injection of the mutagen, which excludes their direct interaction.

In this study, an indirect alkalizing mutagen cyclophosphamide and a prooxidant dioxidine were taken as the "standard" mutagens. The mechanism of damage produced by these agents is similar to that of the majority of known mutagens.

The dose range of the examined modifier was determined on the basis of its possible daily intake by humans. The regimen was not limited by single administration of the examined agents. We found that in addition to acute experiments, it is optimal to study the effect of mutagen administered against the background of treatment with the modifier and the effect of combined daily administration of the mutagen and modifier. In our experiments, the duration of preliminary and combined administration was 5 and 14 days, respectively.

This scheme made it possible to extend our knowledge on antimutagenic activity of β-carotene, aspartame, or their combinations [1,13,25]. Moreover, it helped us to reveal co-mutagenic activity of some chemical agents.

Co-Mutagenic Activity of Drugs

Co-mutagenic activity of medicinal preparations is an important problem because of wide indications and long-term usage of many drugs and high probability of their interference with mutagens. Moreover, in some cases this interference is virtually unavoidable: for example, in complex therapy of cancer and autoimmune diseases or during prescription of routine drugs to persons engaged in unhealthy trades. However, little is known on this type of drug activity *in vivo*. Such papers describe only potentiation of the aneugenic effect of benzene by praziquantel in mice [22] and the presence of antimutagenic and co-mutagenic activities in mucolytic N-acetylcystein [36].

Co-Mutagenic Activity of Calcium Channel Blockers

Calcium antagonists *in vivo* potentiate the mutagenic effects of antitumor drugs in *in vitro* experiments on pro- and eukaryotes [26,33]. These data initiated studies of *in vivo* co-mutagenic activity of these drugs.

The data on antioxidant activity of some calcium antagonists, 1,4-dihydropyridine, benzothiazepine, and phenylalkylamine derivatives [30,37] substantiated the use of pharmacogenetic model employing male C57B1/6 and BALB/c mice contrasting by the phenotype of the antioxidant system [12] in the studies of *in vivo* mutagen-modifying activity of these agents. In general, C57B1/6 mice are more resistant to prooxidant load and to the effects of some mutagens compared to BALB/c mice, albeit initially C57B1/6 mice have lower activity of SOD and catalase [11,18].

The data on lacidipine, a 1,4-dihydropyridine derivative, were published elsewhere (Table 1, [15]). It was found that lacidipine in doses of 0.1 and 1.0 mg/kg moderates the clastogene effect of dioxidine (200 mg/kg) in C57B1/6 mice, while in higher doses (5 and especially 10 mg/kg) it pronouncedly potentates the effect of the mutagen (100 and 200 mg/kg) in both strains.

Both types of mutagen-modifying activity were revealed when lacidipine was used in efficient doses of 0.1–10.0 mg/kg found previously in animal experiments [23].

TABLE 1. Effect of Lacidipine on Clastogenic Effects of Mutagens in Mouse Bone Marrow Cells

Mouse strains	Dioxidine, intraperitoneally	Lacidipine, peroral administration, mg/kg			
		0.1	1	5	10
C57B1/6	Single dose, 100 mg/kg	—	—	+	+
	Single dose, 200 mg/kg	++	++	+	+
	5 days, 100 mg/kg	—	—	n.d.	+
BALB/c	Single dose, 100 mg/kg	—	—	—	—
	Single dose, 200 mg/kg	—	—	(+)	(+)
	5 days, 100 mg/kg	—	—	n.d.	+

Note. +) Significant potentiation of the clastogenic effect; ++) significant attenuation of the clastogenic effect; (+) — mutagen in specified dose demonstrates significant mutagenic effect only in combination with lacidipine; (—) — no significant changes in clastogenic effect; n.d. — no experimental data.

TABLE 2. Effect of Verapamil on Clastogenic Effects of Mutagens in Mouse Bone Marrow Cells

Mouse strains	Mutagen	Verapamil, mg/kg								
		0.1	0.2	0.4	2.5		5		10	
		i.p.	i.p.	i.p.	i.p.	p.o.	i.p.	p.o.	i.p.	p.o.
C57B1/6	Acrylamide 50 mg/kg, i.p., 5 days	n.d.	n.d.	n.d.	n.d.	+	n.d.	—	n.d.	—
	Acrylonitrile 10 mg/kg, i.p., single dose	—	(+)	(+)	(+)	—	—	—	—	—
	5 days	n.d.	n.d.	n.d.	n.d.	(+)	n.d.	(+)	n.d.	—
	Dioxidine 100 mg/kg, 5 days	n.d.	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	+
	Cyclophosphamide 10 mg/kg, i.p., single dose	+	+	+	—	+	+	—	+	—
	5 days	n.d.	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	+
BALB/c	Acrylamide 100 mg/kg, i.p., single dose	—	—	—	—	—	—	+	—	—
	50 mg/kg, 5 days	n.d.	n.d.	n.d.	n.d.	+	n.d.	+	n.d.	—
	Acrylonitrile 10 mg/kg, i.p., single dose	(+)	—	—	(+)	(+)	—	—	—	—
	5 days	n.d.	n.d.	n.d.	n.d.	—	n.d.	—	n.d.	(+)
	Dioxidine 100 mg/kg, 5 days	n.d.	n.d.	n.d.	n.d.	—	n.d.	—	n.d.	—
	Cyclophosphamide 10 mg/kg, i.p., single dose	+	+	—	+	—	+	+	—	—
	5 days	n.d.	n.d.	n.d.	n.d.	—	n.d.	—	n.d.	+

Note. +) Significant increase of the clastogenic effect; (—) — no significant changes of the clastogenic effect; (+) — mutagen in the specified dose demonstrates significant mutagenic effect only in combination with verapamil; n.d. — no experimental data. i.p. — intraperitoneal injection; p.o. — peroral administration.

Antimutagen activity of lacidipine was not surprising: it can be explained from the view point of interrelation between antimutagenic and antioxidant activities of chemical compounds [12] and corresponded to the data of independent studies on drosophila [4]. In turn, the interstrain differences responsible for the observed effects included in the pharmacogenetic model and the dose-dependent divergence of the mutagen-modifying effects suggest that these phenomena can be explained by inversion of antioxidant into prooxidant action characteristic of some antioxidant agents [12,28].

The study of mutagen-modifying activity of calcium blockers was continued with phenylalkylamine derivative verapamil. The original data of this research were reported elsewhere [14-16,32] and generalized in this paper (Table 2).

It was established that peroral or intraperitoneal administration of verapamil in a wide therapeutic dose range of 0.1–10 mg/kg potentiated the effects of four examined mutagens: acrylamide, acrylonitrile, dioxidine, and cyclophosphamide. Verapamil demonstrated some specificity of co-mutagenic modification in experiments with various mutagens and this modification depended on the experimental protocol (duration and mode of administration). Co-mutagenic activity of verapamil was more pronounced after repeated administration, and in some experiments it depended on mouse strain. However, all these peculiarities are only quantitative, while the whole data attest to co-mutagenic properties of verapamil towards four test mutagens.

There were no quantitative differences in the co-mutagenic effects of the examined calcium channel blockers. The increase in the number of cells with aberrant chromosomes under the effect of lacidipine or verapamil used in therapeutic doses was 50-120% irrespective of the applied mutagen and depended only on the regimen of administration.

Therefore, representatives of two of three known groups of calcium antagonists demonstrated co-mutagenic activity *in vivo*. It is possible that this undesirable activity is characteristic of all drugs of this class of pharmacological agents. This hypothesis could be verified by the tests for co-mutagenic activity of calcium antagonists belonging to benzothiazepine derivatives. However, the established co-mutagenic activity of lacidipine and verapamil necessitates clinical study of their co-mutagenic properties during complex therapy in combination with the drugs with established or suspected mutagenic activity (these drugs were listed elsewhere [12]). This study could be based on efficient procedures of cytogenetic analysis in combination with DNA-comet assay.

Co-Mutagenic Activity of Food Additives

Food is the main source of mutagens for human organism. Food can contain environmental mutagens (heavy metals, pesticides, and other ecotoxic agents) or muta-

gens formed during thermal processing or storage. The data on food mutagens were reviewed previously [6, 9]. Little is known on food co-mutagens, although the first co-mutagens harman and norharman were found in food. Moreover, there are almost no data on *in vivo* co-mutagenic activity of food components except vitamins and some flavonoids [12].

Food additives. There is a moiety of food additives of various types, which are not necessary ingredients of human diet [3]. Under the framework of integral program with Research Institute of Brewing, Soft Drinks, and Wine Industry of Russian Academy of Agricultural Sciences, we studied some food additives used in the production of drinks. Specifically, co-mutagenic activity was revealed in food dye Sunset Yellow FCF (E-110) widely used in European Community and Russia (Tables 3 and 4).

The effect of E-110 dye on clastogenesis induced by dioxidine or cyclophosphamide in bone marrow cells was studied on mice. The dye was administered perorally in doses of 0.1, 1, and 2.5 mg/kg. This dose range approximately corresponds to daily consumption of the dye in humans [3].

It was found, that Sunset Yellow in single and preliminary administration in a minimum dose of 0.1 mg/kg significantly increased (by 1.5-1.8 times) the yield of aberrant cells under the action of dioxidine. Similar effect was observed when the dye was used in a single dose of 1 mg/kg (Table 3).

Even more pronounced co-mutagenic activity of E-110 was revealed in the experiments with cyclophosphamide. When used in minimal and intermediate single doses, the dye significantly increased the yield of aberrant cell (by 1.2–1.5 times) affected by this mutagen. The dye in all doses (preliminary and repeated 5-fold administration) 1.7-2.7-fold potentiated the clastogenic effect mutagen (Table 4).

It should be emphasized that the co-mutagenic effect of Sunset Yellow was reproduced during independent repeated experiments. However, there were two cases, when single administration of the dye in doses of 1 or 2.5 mg/kg significantly reduced the clastogenic effect of cyclophosphamide. These single observations seem not to be artifacts, since they reflect general tendency explained by co-mutagenesis mechanisms.

Published data suggest [12] that caffeine widely used in many food products and soft drinks as a food additive can potentiate the effect of various mutagens in somatic and germ mammal cells, when it is applied in doses of 4 to 200 mg/kg.

Vitamins. Various aspects of the study of mutagen-modifying potency of vitamins were considered in details [5,12,19]. The analysis concluded that experimental data attesting to antimutagenic, co-mutagenic, and even mutagenic effects of vitamins (first of all, antioxidant vitamins) should not be extrapolated to humans. In contrast to laboratory rodents, humans cannot produce vitamin C, which in combination with other antioxidant vitamins and some low-molecular weight antioxidant agents forms non-enzymatic part of the antioxidant system. Therefore, the humans and laboratory test-model animals are principally different in this respect. It means that the use of animals (except guinea pigs and some other animals that cannot synthesize vitamin C) in the studies of the biological effects of antioxidant vitamins is not reasonable due to ambiguity of the results.

The data obtained on human cell cultures are more reliable, but not perfect because most data were obtained with vitamin far exceeding their real content in the organism.

Thus, the mutagen-modifying properties of vitamins can be objectively assessed only on the basis of very limited number of papers, which study sensitivity of human cells to mutagenic action in dependence on vitamin status. These papers were reviewed elsewhere [5,12].

So far, evaluation of the effect of various factors on hereditary variability in humans was performed by comparison of group mean indices, while there are data attesting to possible genotypic and phenotypic determinism of the hereditary variability [12], which should be compared only on individual basis. This prerequisite was taken into consideration in two reported studies devoted to the analysis of group and individual changes in cytogenetic variability under the effect of dioxidine and bleomycin, which were added to donor cell culture before and after additional intake of complex vitamin preparations of various quantitative and qualitative compositions [8,17].

It was found that two-week intake of a vitamin-mineral complex containing virtually all essential nutrients in doses 2-3-fold surpassing the necessary daily dose improved the resistance of peripheral blood lymphocytes of healthy donors (n=15) to the cytogenetic effect of bleomycin (1.0 U/ml) and dioxidine (0.1 mg/ml). However, this treatment did not modulate the effects of mutagens applied in doses of 0.1 U/ml and 0.01 mg/ml, respectively. Moderation of the clastogenic effect was detected after comparison of the number of the cells with aberrant chromosomes in different groups (mean values) and in different donors (p<0.05). There were no cases with potentiation of the mutagenic effects [17].

Other picture was observed after intake of a vitamin complex, which was qualitatively similar, but contained nutrients in doses corresponding to their daily consumption level. This treatment decreased the resistance of cells to the clastogenic effect of dioxi-

dine in a dose of 0.1 mg/ml (but not 1.0 mg/ml). Cell sensitivity to bleomycin (0.1 and 1.0 U/ml) significantly increased in some donors and decreased in others ($p<0.05$) without significant changes in the mean indices for the group [8]. The latter result suggests that in some individuals additional intake of vitamins can increase cell vulnerability to some mutagens. In other words, high doses of vitamins can produce a co-mutagenic effect.

Possible Mechanisms of Co-Mutagenic Activity

Unfortunately, the data describing co-mutagenic mechanisms are scarce. General observations suggest that in overwhelming majority of cases the mechanisms of co-mutagenic action are reversal to the antimutagenic mechanisms. The latter were considered in details in some papers [12,24,36]. For instance, some substances exert antimutagenic action via improvement of the repair processes. Probably, the agents inhibiting this process would exhibit co-mutagenic activity. The co-mutagenic mechanisms can be related to 1) inversion of antioxidant effect into prooxidant action with concurrent inhibition of the antioxidant system, 2) modulation of metabolic systems, 3) modification of apoptosis, and 4) some other general biological processes.

In addition to specified general mechanisms of co-mutagenesis, specific mechanisms can also exist, which are not the mirror reflection of the antimutagenic mechanisms. In particular, calcium channel blockers seem to exert co-mutagenic action in two ways. First, they act via general mechanisms modulating activity of microsomal enzymes, free radical oxidation [14,20,32], and cell proliferation [29,34]. Second, they can specifically affect function of P-glycoprotein, which control penetration of cytostatics into cells [32,33]. It cannot be excluded that co-mutagenic activity of these drugs can be caused by their specific effect on calcium metabolism, although this

TABLE 3. Effect of Food Dye Sunset Yellow on Clastogenic Effects of Dioxidine in CDF_1 Mice

Experiment	Cell number	Per 100 examined cells					Cells with aberrant chromosomes ($M\pm m$, %)
		gaps	single fragments	paired fragments	exchanges	cells with multiple abberations	
Control	500	1.2	2.0	0	0	0	3.2±0.8
Acute experiment, single dose							
Dioxidine							
200 mg/kg	500	1.2	3.6	0.2	0.8	3.2	8.4±1.2
+Sunset yellow							
0.1 mg/kg	500	0.8	9.0	0.8	0.4	3.4	12.4±1.5**
1 mg/kg	400	1.4	7.5	0.3	0.8	5.3	13.3±1.7**
2.5 mg/kg	500	0.6	5.8	0.2	0.2	4.8	10.8±1.4
Preliminary treatment with dye for 5 days, mutagen in a single dose with the last administration of the dye							
Dioxidine							
200 mg/kg	500	1.2	3.6	0.2	0.8	3.2	8.4±1.2
+Sunset yellow							
0.1 mg/kg	400	0.5	8.8	—	1.8	5.5	15.3±1.8*
1 mg/kg	500	0.8	3.6	0.6	1.2	1.2	7.0±1.1
2.5 mg/kg	500	1.0	5.2	0.4	—	4.6	10.2±1.4
Combined administration, 5 days, C57B1/6 mice							
Dioxidine							
200 mg/kg	400	2.5	9.0	—	—	3.3	12.8±1.7
+Sunset yellow							
0.1 mg/kg	500	1.2	5.8	0.6	0.6	2.0	9.0±1.2
1 mg/kg	400	1.5	9.0	0.5	—	3.8	12.3±1.6
2.5 mg/kg	400	0.5	6.8	0.3	0.5	3.0	9.0±1.4

Note. Here and in Table 4: *$p<0.01$, **$p<0.05$ in comparison with other mutagen.

TABLE 4. Effect of Food Dye Sunset Yellow on Clastogenic Effects of Cyclophosphamide in CDF_1 Mice

Experiment	Cell number	Per 100 examined cells					Cells with aberrant chromosomes ($M\pm m$, %)
		gaps	single fragments	paired fragments	exchanges	cells with multiple abberations	
Control	500	1.2	2.0	0	0	0	3.2±0.8
Acute experiment, single dose							
Cyclophosphamide							
20 mg/kg	400	1.5	18.0	0.5	0.5	10.0	25.0±2.2
+Sunset yellow							
0.1 mg/kg	500	0.8	26.4	1.0	2.2	17.2	36.6±2.1*
1 mg/kg	500	2.2	19.6	0.8	1.6	12.8	30.2±2.1**
2.5 mg/kg	500	0.4	20.8	0.2	2.0	10.0	26.6±2.0
Preliminary treatment with dye for 5 days, mutagen in a single dose with the last administration of the dye							
Cyclophosphamide							
20 mg/kg	500	0.8	10.4	—	1.4	1.4	12.4±1.5
+Sunset yellow							
0.1 mg/kg	500	1.8	19.2	0.8	0.6	3.6	20.4±1.8*
1 mg/kg	500	0.8	17.8	0.2	2.4	17.4	33.6±2.1*
2.5 mg/kg	500	0.8	21.8	0.6	2.2	2.2	21.2±1.8*
Combined administration, 5 days, C57B1/6 mice							
Cyclophosphamide							
20 mg/kg	400	1.5	10.5	—	1.5	—	13.0±1.7
+Sunset yellow							
0.1 mg/kg	500	3.4	16.0	0.4	1.4	10.4	27.0±2.0*
1 mg/kg	500	4.6	25.6	1.0	0.8	2.4	27.6±2.0*
2.5 mg/kg	400	5.3	22.0	—	—	1.2	23.0±2.1*

assumption contradicts formally to the previous hypothesis that intracellular accumulation of calcium is related to DNA aberrations during oxidative stress [27].

The detailed study of the mechanisms of co-mutagenic activity of calcium channel blockers needs special experimental work. At present, it can be assumed that the mechanism related to modification of mutagen metabolism is hardly possible, since the study revealed potentiation of mutagenic activity of dioxidine, which is not subjected to biological conversion (Tables 1 and 2).

Experience accumulated during the study of antimutagenesis showed that the protective effects of antimutagens are rarely realized via a single mechanism. It is highly possible that co-mutagens also exert their negative influence in a polyvariant mode.

Summing up our observation and published data, three peculiarities should be pointed out: high probability to reveal co-mutagenic effect in subacute experiments, the absence of pronounced dose-dependences of its manifestation, and frequent combination of co-mutagenic and antimutagenic activity in the same agent. Future study will show, whether these features are universal for the action of co-mutagens *in vivo* or they characterize only the substances examined in our work.

Conclusion

A question arises on how actual is the problem of co-mutagenesis. The definite answer can be obtained only by special epidemiological studies. However, the above-mentioned facts attest to potentiation of genotoxic effects by combined action of mutagens and co-mutagens. Therefore, the use of co-mutagens in the presence of environmental pollutants is problematic. The co-mutagenic properties should be considered as a limiting factor to the use of drugs, nutrients, and other chemical compounds providing adequate replacement of these agents with substances exhibiting no co-mutagenic activity is possible. Therefore, the search and study of co-mutagenic agents should yield recom-

mendation for application of co-mutagenic substances, which should extend current requirements for genotoxic tests.

We believe that the existence of *in vivo* co-mutagenic activity in some widely used substances, *e.g.* hypotensive drugs and food additives, substantiates and necessitates the search and study of possible co-mutagens. This task is not very complex, because administratively it suggests introduction of additional requirements to the existing norms on genotoxic screening of new synthesized substances and scientifically it can use the reported principles of examination. Detection of co-mutagens and the control over their use would help to reduce the negative effects of induced mutagenesis on human health.

To solve these problems, it is necessary to take reasonable steps. For example, novel calcium channel blockers should be tested for co-mutagenic activity. First of all, such tests should be performed for diltiazem derivatives with assessment of their interaction with a broad spectrum of mutagens including ionizing radiation and the study of the mechanisms of co-mutagenic effects. The latter can be of practical interest, since it would show the ways to preventing the harmful effects. However, realization of these measures can be of practical importance only after corroboration of the co-mutagenic action of calcium channel blockers in humans. A wide clinical and outpatient use of this class of drugs opens new vistas in medical cytogenetic studies that can elucidate this problem.

In light of massive consumption of vitamins under the pressure of widely advertised food additives, even single facts indicating the possibility of reducing tolerance to mutagenic effects by these agents are very important and alarming. Another alarming fact is wide spread of various biological flavonoids, which are often used together with vitamins. Complexity of studies of bioactive additives results from heterogeneity of their quantitative and qualitative compositions, differences in bioavailability of their components in advertised brands, genetic heterogeneity of consumers, differences in their initial supply of vitamins, and their lifestyles. The absence of adequate biological test-systems should be added to this list. Probably, the most efficient approach to this problem is simultaneous assessment of several biological markers of mutagenesis characterizing its level in various tissues and parallel evaluation of vitamin and antioxidant statuses in epidemiological trials.

In conclusion, it should be noted that the phenomenon of co-mutagenesis, which aggravate the genetic load on living organism, could be considered as a potentiating effect from pharmacological viewpoint. The study of co-mutagenesis from this point can be useful for chemotherapy of malignant tumors.

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