Cytotoxic Effect of Flavonoids on Leukemia Cells and Normal Cells of Human Blood

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We compared the cytotoxic effect of 11 flavonoids on chronic myeloid leukemia (erythroblast crisis) K562 cells and peripheral blood mononuclear cells from healthy donors. Baicalein and myricetin had a specific cytotoxic effect on leukemia cells.

Key Words: *flavonoids; cytotoxic effect; chronic myeloid leukemia; peripheral blood mononuclear cells; phytohemagglutinin*

Low specificity of drug chemotherapy is an important problem of oncology, because it determines the development of side effects in patients. The search for efficient and strongly specific anticancer drugs is an urgent problem. Previous studies showed that some flavonoids have specific cytotoxic activity in relation to malignant cells of different etiology [5]. Much attention was paid to the effect of various flavonoids on tumor cells. However, the influence on cultured normal cells was evaluated only for some compounds (*e.g.*, quercetin and flavopiridol) [2,3].

Here we studied the efficacy and specificity of cytotoxic action of flavonoids on leukemia cells and normal blood cells.

MATERIALS AND METHODS

The K562 cell line was cultured in RPMI 1640 medium with 10% fetal bovine serum, 4 mmol/liter L-glutamine, 1.5 g/liter sodium bicarbonate, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humid atmosphere at 5% CO_2 and 37°C.

Peripheral blood mononuclear cells (PBMC) were isolated by the standard method on a Ficoll-Verografin density gradient (1.077 g/liter) and incubated in complete nutrient medium RPMI 1640 (humid atmosphere, 5% CO_2). Blast transformation of PBMC was induced by addition of phytohemagglutinin (PHA) in a concentration of 5 μg/ml to the culture medium.

For cytotoxic tests, the cells were incubated in 96-well culture plates for 96 h. The test flavonoids in concentrations of 5, 10, 20, 40, 80, 160, and 320 μmol/liter were added (except for the control well). The initial concentration of K562 cells and PBMC was 10^5 and 10^6 cells/ml, respectively. The MTT test was performed routinely [4]. Each experiment was conducted in at lest 6 repetitions.

IC_{50} was estimated from the results of the MTT test (nonlinear regression analysis) as described elsewhere [1]. The regression analysis was conducted using OriginPro 7.0 software.

The results were analyzed by Student's *t* test (OriginPro 7.0 software). The data are expressed as the arithmetic mean and standard error of the mean.

RESULTS

Experiments were performed with the following three types of cells: chronic myeloid leukemia (erythroblast crisis) K562 cells; freshly isolated PBMC from healthy donors; and PHA-stimulated (*in vitro* blast transformation after treatment with PHA) PBMC from healthy donors (model for the pool of dividing immature human blood cells). The mean degree of cell stimulation with PHA was 77%. Flavonoids dose-dependently decreased viability of leukemia cells and PBMC from

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healthy donors (with or without blast transformation; Fig. 1).

IC_{50} was estimated from the dose-response curves in each experiment (MTT test; Table 1). The coefficient C1 reflects the selective effect of the test flavonoid on K562 leukemia cells as compared to PHA-unstimulated PBMC (Table 1). This coefficient was calculated as the ratio of IC_{50} for the effect of the test flavonoid on PHA-unstimulated PBMC and K562 cells. The coefficient C2 reflects a selective effect of the test flavonoid on K562 leukemia cells as compared to PHA-stimulated PBMC. This coefficient was calculated as the

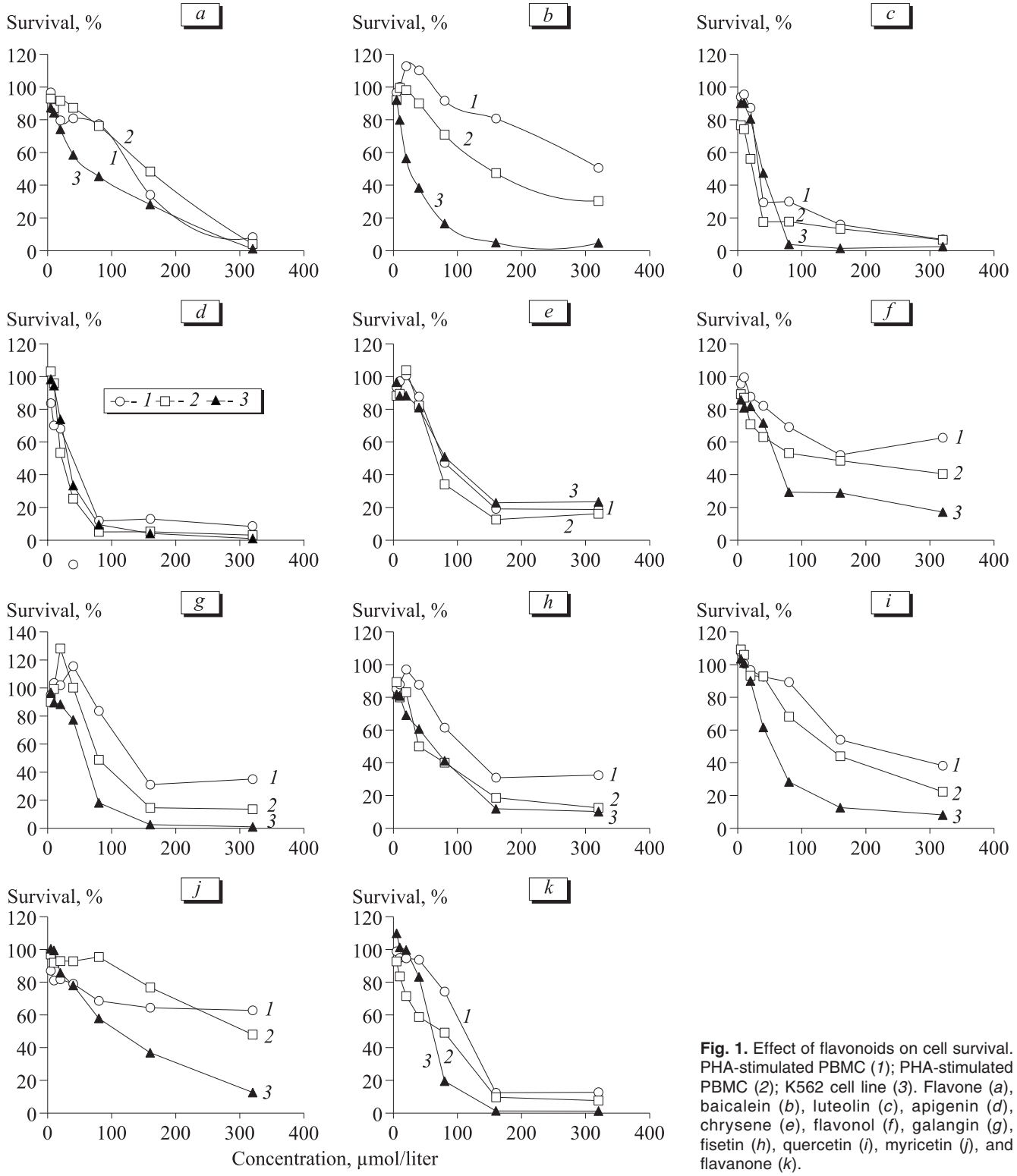

Fig. 1. Effect of flavonoids on cell survival. PHA-stimulated PBMC (*1*); PHA-stimulated PBMC (*2*); K562 cell line (*3*). Flavone (*a*), baicalein (*b*), luteolin (*c*), apigenin (*d*), chrysene (*e*), flavonol (*f*), galangin (*g*), fisetin (*h*), quercetin (*i*), myricetin (*j*), and flavanone (*k*).

TABLE 1. Parameters of Cytotoxic Effect of Flavonoids (*M*±*m*)

Active compound	IC_{50}, μmol/liter			Relative coefficients		
	K562 cell line	PHA-unstimulated PBMC	PHA-stimulated PBMC	C1	C2	C3
Flavones						
flavone	80.1±1.3	114.8±18.8	136.5±17.8	1.43	1.70	0.84
baicalein	24.3±2.5	319.6±36.6*	160.2±12.6*	13.15	6.59	2.00
luteolin	30.7±2.0	38.2±6.9	20.7±3.5	1.24	0.67	1.85
apigenin	36.5±4.9	29.2±4.8	23.5±1.7	0.80	0.64	1.24
chrysene	59.4±5.5	84.4±9.2	67.4±8.9	1.42	1.13	1.25
Flavonols						
flavonol	49.8±8.0	260.8±53.4*	130.2±20.3*	5.24	2.61	2.00
galangin	44.3±2.0	137.7±20.2*	82.8±12.9*	3.11	1.87	1.66
fisetin	62.9±3.4	122.2±24.6*	66.9±6.1	1.94	1.06	1.83
quercetin	64.1±5.1	212.9±22.8*	139.1±13.8*	3.32	2.17	1.53
myricetin	78.2±4.8	1192.2±456.6*	310.8±35.8*	15.25	3.97	3.84
Flavanones						
favanone	55.1±4.0	102.8±7.3*	51±7.6	1.87	0.93	2.02

Note. *$p<0.05$ compared to the K562 cell line.

ratio of IC_{50} for the effect of test flavonoid on PHA-stimulated PBMC and K562 cells. The coefficient C3 reflects the dependence of flavonoid cytotoxicity on proliferative properties of target cells. This coefficient was calculated as the ratio of IC_{50} for the effect of the test flavonoid on PHA-unstimulated PBMC and PHA-stimulated PBMC. These data allow us to evaluate the specificity of various flavonoids in relation to malignant and normal cells or proliferating and nonproliferating blood cells. The probability for *in vivo* side effects of test flavonoid can be also evaluated.

The specificity of flavonoids in relation to 3 types of cells was shown to vary significantly (Table 1). For example, IC_{50} of flavone, apigenin, chrysene, and luteolin did not differ for each type of target cells. Fisetin and flavanone had a smaller cytotoxic effect on PHA-unstimulated PBMC from healthy donors (by 1.5-2 times). By contrast, the action of these compounds on PHA-stimulated PBMC was similar to the cytotoxic effect on K562 cells. The cytotoxic effect of other flavonoids on leukemia cells was greater than that on normal blood cells. It should be emphasized that blast-transformed cells were more sensitive to the effect of flavonoids than PHA-unstimulated PBMC. The highest specificity of anti-leukemia action was typical of baicalein and myricetin. Therefore, these flavonoids hold much promise (as compared to other substances of this group) for the development of new chemotherapeutics in chronic myeloid leukemia. We conclude that the majority of flavone compounds (except for baicalein) have greater cytotoxic activity, but lower specificity of the anti-leukemia effect than flavonol substances.

Our results indicate that flavonoids do not produce specific inhibitory effect on leukemia cells, but also affect normal blood cells in a dose-dependent manner. However, significant differences were revealed in the cytotoxic effect of some flavonoids on leukemia cells and normal cells. Further studies are required to evaluate the possibility of chemotherapy with these compounds.

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