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SHORT COMMUNICATION

SYNTHESIS AND TUMORICIDAL ACTIVITY EVALUATION OF NEW MORIN AND QUERCETIN SULFONIC DERIVATIVES

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Abstract: Flavonoids are a group of naturally occurring compounds with interesting medical properties, such as antiinflammatory, antiallergic, antiviral, antibacterial and antitumor activities. In our experiments we were trying to examine the tumoricidal activity of newly synthesized derivatives of two flavonoids: 3,5,7,2',4' - pentahydroxyflavone (morin) and 3,5,7,3',4' - pentahydroxyflavone (quercetin). These derivatives were: natrium salt of morin-5'-sulfonic acid (NaMSA), natrium salt of quercetin-5'-sulfonic acid (NaQSA), complex of Mg^{2+} with quercetin-5'-sulfonic acid (QSA), complex of iron(II) with QSA. The antitumor activity of these agents was tested *in vitro* on two cell lines: L1210 - murine lymphocytic leukaemia and P-815 - murine mastocytoma. Our experiments showed that sulfonic derivatives of these two flavonoids were less potent than the original agents in their cytostatic and cytotoxic activities. However, their solubility in water was greater than that of the original agents and higher culture medium concentration of these derivatives was obtained. The results indicate that the ability of flavonoids to act tumoricidally is reciprocally correlated with their lipophilicity.

Keywords: morin, quercetin, quercetin and morin sulfonic derivatives, cytostatic, cytotoxic activities

Flavonoids, derivatives of benzo-γ- pirone, are a group of widely distributed compounds in the plant kingdom. The family includes monomeric flavanoles, flavanones, anthocyanidins, flavones and flavonoles. Individual differences within each group result from the variation in number and arrangement of the hydroxyl groups as well as from the nature and extend of alkylation and/or glycosidation of these groups. The polyphenolic components of higher plants may act as antioxidants or as agents of other mechanisms contributing to anticarcinogenic, antiviral, antibacterial, antiinflamatory and cardioprotective actions (1-3). It was also well established that there are many structure - activity relationship within this group of compounds. It is well known that the most potent of all polyphenols are the aglycones but they are also less soluble in water. That is why many investigators are searching for synthetic derivatives, which have the same activity as aglycone but better water solubility.

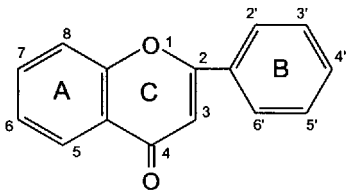
In our experimental model we compared the antitumor activity of morin and quercetin with their sulfonic derivatives alone and in complexes with iron (II) and magnesium (II). The pattern of antitumor efficacy of five tested flavonoids and their sulfonic derivatives was compared with the cytosine arabinoside, an established cytostatic drug.

EXPERIMENTAL

Chemicals

Morin (3,5,7,2',4'-pentahydroxyflavone, $C_{15}H_{10}O_7 \cdot 2H_2O$) and quercetin (3,5,7,3',4'-pentahydroxyflavone, $C_{15}H_{10}O_7 \cdot 2H_2O$) were obtained from Carl Roth KG Karsruhe, Germany. Natrium salt of morin-5'-sulfonic acid - $C_{15}H_9O_{10}SNa \cdot 2H_2O$ (NaMSA) and natrium salt of quercetin-5'-sulfonic acid - $C_{15}H_9O_{10}SNa \cdot 4H_2O$ (NaQSA), complex of magnesium(II) with quercetin-5'-sulfonic acid (QSA) - $Mg(C_{15}H_9O_{10}S)_2 \cdot 8H_2O$, complex of iron(II) with QSA - $Fe(C_{15}H_9O_{10}S)_2 \cdot 8H_2O$ were synthesized as described previously [4-8]. Figure 1 shows the structure of quercetin and morin and their sulfonic derivatives. Structure of the complexes are not shown, they are still investigated. Sulfonic derivatives as well as their complexes with metals have good water solubility (about 10^{-2} M).

Cytosine arabinoside (Cytosar), was purchased from Upjohn s.a. PUUrs - Belgium.



Name	3	5	7	2'	3'	4'	5'
Quercetin	OH	OH	OH	H	OH	OH	H
Morin	OH	OH	OH	OH	H	OH	H
QSA	OH	OH	OH	H	OH	OH	SO_3H
NaQSA	OH	OH	OH	H	OH	OH	SO_3Na
NaMSA	OH	OH	OH	OH	H	OH	SO_3Na

Figure 1. The structure of quercetin and morin and their sulfonic derivatives.

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) was purchased from Sigma Chemical Co., St. Louis USA.

Cell lines

Two cancer cell lines: L1210 – murine lymphocytic leukaemia and P-815- murine mastocytoma were employed (a generous gift from Prof. C. Radzikowski, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland). Both lines grew in suspension culture, partly floating and partly attached, in RPMI 1640 medium (GIBCO BRL Life Technologics Ltd., Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum, 25 mM HEPES buffer, 2 mM L-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in a humidified atmosphere of 5% CO_2 in air. Cytotoxicity after treatment of the tumor cells with the test compounds was determined using the microculture tetrazolium (MTT) assay (9–11). This assay is based on the reduction of a soluble tetrazolium salt by mitochondrial dehydrogenase in viable cells into an insoluble coloured formazan product, which can be measured spectrophotometrically after dissolution.

Cell viability quantification

After addition of the tested agent to cell cultures, the cells were incubated for 24 h or 6 days with an appropriate drug in a CO_2 incubator (5% CO_2 + 95% air) before the MTT reagent was added. The tested flavonoids were studied at concentrations: quercetin and morin – 10 – 50 µM, sulfonic

Table 1. The comparison of LD_{50} drug's concentrations from all experiments. The data are means of four independent experiments ± SD.

Name of the compound	LD_{50} [µmol/l][1]			
	P-815 [2]		L_{1210} [3]	
	24 h[4]	6-day[5]	24 h	6-day
Quercetin	> 50	5.0±0.8	47±6	< 1
Morin	> 50	> 50	44±5	> 50
NaQSA	> 200 (360)	> 200 (220)	> 200	107±13
NaMSA	> 200	> 200	> 200	> 200
$Mg(QSA)_2$	> 200 (255)	> 200	> 200	149±18
$Fe(QSA)_2$	> 200	> 200	> 200	87±10
Cytosine arabinoside	1.00±0.08	0.020±0.001	2.0±0.2	0.040±0.003

1) – concentration of drug that produce 50% reduction of absorbance – viability of neoplastic cells
2) – murine mastocytoma cell line
3) – murine lymphocytic leukemia cell line
4) – 24-h incubation of neoplastic cells with appropriate drug
5) – 6-day incubation of neoplastic cells with appropriate drug

derivatives 10 – 200 μM (4 repetitions of each sample). Drugs effect was quantitated as the percentage of control absorbance of reduced dye at 550 nm. The absorbance was determined with an automated microplate reader Elx 800 (Bio–Tek Instruments Inc. Winooski, VT, USA). The LD_{50} was defined as the concentration of drug that produce 50% reduction of absorbance.

RESULTS AND DISCUSSION

Flavonoids show diverse biological activities, some of them related to antitumor activity (11–17).

The two flavonoles and their derivatives were evaluated for cytotoxic (24 h incubation) and cytostatic (6–day incubation) activities in two cancer cell lines: P–815– murine mastocytoma and L1210 – murine lymphocytic leukaemia.

Obtained results are summarized in Table 1. In our experiments we confirmed higher cytotoxic and cytostatic activities of quercetin and morin than their derivatives against two tested cancer cell lines. Morin has less potent antitumor activity against two cell lines during 6–day incubation. The cytotoxic and cytostatic activity of sulfonic derivatives was almost completely abolished or at least markedly reduced comparing with original agents. Against L1210 cancer cell line higher cytostatic activity of quercetin sulfonic derivatives was observed. It is worth noting that both complexes of iron (II) with QSA are stable (Fe^{2+} does not transit to Fe^{3+}). The cytosine arabinoside as a model antitumor drug to compare with the tested compounds was used.

This study shows that quercetin and morin are potent cytotoxic compounds against P–815 and L1210 cells. Quercetin is known to display a variety of biological actions and numerous studies have reported its powerful growth inhibitory activity *in vitro* on various tumor cells (2).

In determining the possible structure–activity relationship of the investigated flavones, one should consider not only the number of the hydroxy group and their position in the benzopyrone ring, but also the hydrophobic/hydrophilic nature of each compound (18–21).

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