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STRUCTURAL BASIS FOR ANTIVIRAL ACTIVITY OF FLAVONOIDS - NATURALLY OCCURRING COMPOUNDS

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Summary. — The effect of different substituents of quercetin and luteolin on the ability to inhibit the herpes simplex virus (HSV-1) replication in RK-13 cells was studied. It seems that parent compounds with free hydroxyl groups at C-5, C-7, C-3′, C-4′ and additionally at C-3 have the highest activity. Substitution of those groups caused decrease of or completely abolished the antiviral activity of the tested compounds.

Key words: flavonoids; antiviral activity, herpes simplex virus

Introduction

Flavonoids are a group of natural products exhibiting a broad biological activity (Havsteen, 1983). Some of them display potent antiviral effects both *in vitro* and *in vivo* (Béládi *et al.*, 1981); flavonoids such as rutin, hesperidin, citrus bioflavonoids complex are used in the therapy of viral diseases (Verbenko *et al.*, 1979; Berenge and Exposite, 1975). Independently of clinical trials, investigations on the activity of other flavonoids have been carried out. Kaul *et al.* (1985) showed lately important structure-activity relationship in this group of natural compounds. It seemed interesting to test the antiviral activity of flavonoids with the special emphasis on the effect of substituents.

Materials and Methods

Flavonoids. Quercetin, rhamnetin, celastrozide, herbacetin, rutin, pentamethylquercetin, quercitrin, quercimeritrin, quercetagitrin, phelodendrozide, luteolin, 7-glucose luteolin and naringin (Fig. 1, Table 1) were obtained from the Department of Biology and Pharmaceutical Botany of Medical Academy in Wroclaw. The flavonoids were dissolved in dimethylsulfoxide (DMSO) and then were dilluted with Eagle's minimal essential medium (EMEM) to obtain the indicated concentrations.

Virus. Herpes simplex virus type 1 (HSV-1) was maintained in RK-13 cells. Stock virus titre was $10^{6.3}$ $TCID_{50}$ measured in RK-13 cells.

Inhibition of virus multiplication. RK-13 cells in tubes were infected with 100 $TCID_{50}$ of herpes simplex virus. After 1 hr adsorption at 37 °C the cells were washed twice with Eagle's solution and fed with a medium containing different concentration of the tested flavonoids

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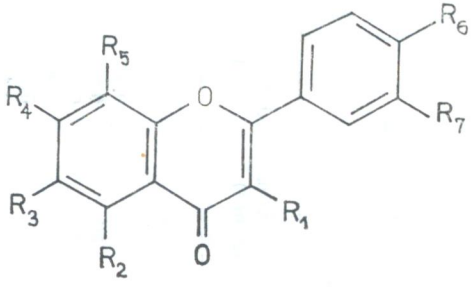

Fig. 1.
The basic structure of flavonoids
For radical substitutions see Table 1

(10, 25, 50, 100 μg/ml). The cells were incubated for 24 hr and then the virus yield was determined. Appropriate DMSO controls were included to monitor the nonspecific reduction of the HSV-1 titre.

Results and Discussion

The results indicate that some of the tested flavonoids possess a significant antiviral activity confirming the earlier observations (Béládi *et al.*, 1977; Béládi et al., 1981; Kaul *et al.*, 1985). Among the flavonoids tested, quercetin with five hydroxyl groups had the highest activity (Table 1). Other compounds inhibited the propagation of HSV-1 to a different extent but none of them showed higher activity than that of quercetin. Phelodendrozide, pentamethylquercetin, herbacetin, rhamnetin and rutin had no activity at all. All these compounds contain a glucose moiety or methyl group. The lack of the activity of pentamethylquercetin was surprising in view of the potent antimicrobial and antifungal effects of lipophylic compounds with several methyl groups (O'Neill and Mansfield, 1982).

Additional hydroxyl group at C-6 had no influence on the observed activity. Substitution of hydroxyl group with a sugar moiety led to a decrease of or even completely abolished the antiviral effect. Presence of OH group at C-3 was not necessary since luteolin (flavon) showed the highest activity of the tested compounds. Similarly to quercetin derivatives, introduction of sugar moiety or methyl group to luteolin resulted in a decrease of the antiviral activity.

From our experiments it seems that the compounds with free (unsubstituted) hydroxyl groups at C-5, C-7, C-3′, C-4′ such as luteolin and additionally at C-3 such as quercetin, inhibited the propagation of HSV-1 to the greatest extent. Substitution of those groups led to a decrease of or completely abolished antiviral effect of the tested compounds.

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References

Béládi, I., Pusztai, R., Mucsi, I., Bakay, M., and Gábor, M. (1977): Activity of some flavonoids against viruses. *An. N.Y. Acad. Sci.* **284**, 358–364.

Béládi, I., Mucsi, I., Pusztai, R., Bakay, M., Rosztóczy, I., Gábor, M., and Veckenstedt, A. (1981): *In vitro* and *in vivo* antiviral effects of flavonoids, pp. 443–450. *Proc. Intern. Bioflav. Symp.* Munich, F.R.G.

Table 1. The effect of flavonoids on herpes simplex virus multiplication in RK13 cells

Flavonoid	R_1	R_2	R_3	R_4	R_5	R_6	R_7	Concentration (μg/ml)[a]	Inhibition (log)[b]
Quercetin	OH	OH	H	OH	H	OH	OH	100	1.34
Rhamnetin	OH	OH	H	OCH_3	H	OH	OH	50	0.17
Celastrozide	O-rham	OH	H	O-rham	H	H	H	100	0.67
Herbacetin	OH	OH	H	OH	O-glc-glc	OH	OH	50	0.00
Rutin	O-glc-rham	OH	H	OH	H	OH	OH	50	0.17
Pentamethylquercetin	OCH_3	OCH_3	H	OCH_3	H	OCH_3	OCH_3	100	0.00
Quercitrin	O-rham	OH	H	OH	H	OH	OH	25	0.90
Quercimeritrin	OH	OH	H	O-glc	H	OH	OH	50	0.44
Quercetagitrin	OH	OH	OH	O-glc	O-glc	OH	OH	100	0.67
Phelodendrozide	O-glc	OH	H	OH	C_5H_9	OH	H	100	0.00
Luteolin	H	OH	H	OH	H	OH	OH	50	2.34
7-glc-luteolin	H	OH	H	O-glc	H	OH	OH	50	0.90
Naringin	H	OH	H	OH	H	OCH_3	OH	100	1.44

[a]) concentration giving the highest decrease of virus titre as compared with control
[b]) difference between control and treated infected cells; titre in the control was $10^{6.23}$ $TCID_{50}$

Berenge, A., and Exposite, R. (1975): A double-blind trial of (+)-cyanidol-3 in viral hepatitis, pp. 201—203. In *New trends in the therapy of liver diseases*, Intern. Symp. Tirrena, Karger, Basel.

Havsteen, B. (1983): Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **32(7)**, 1141—1148.

Kaul, T. N., Middleton, J., and Ogra, P. L. (1985): Antiviral activity of flavonoids on human viruses. *J. med. Virol.* **15**, 71—79.

O'Neill, T. M., and Mansfield, J. W. (1982): Antifungal activity of hydroxyflavans and other flavonoids. *Trans. Br. mycol. Soc.* **79(2)**, 229—237.

Verbenko, E., Petrova, I., Goncharova, L., and Dudarev, A. (1979): Chelepine treatment of some viral diseases of the skin. *Vest. Dermatol. Venerol.* **6**, 51—53.

Book Review

Nongenotoxic Mechanisms in Carcinogenesis

B. E. Butterworth, T. J. Slaga (Eds): Branbury Report 25: Nongenotoxic Mechanisms in Carcinogenesis, pp. 397, Cold Spring Harbor Laboratory, 1987, price $ 70,00.

Knowledge in chemical carcinogenesis clearly demonstrates that cancer development in many tissues and organs is a multifactorial and multistep process. In animal carcinogenesis at least three stages: initiation, promotion and progression are involved. An assumption generally accepted over the last decade was that an alteration of DNA is involved in initiation. To identify environmental carcinogens, various long and short term assays were performed, many of which measure genotoxicity of the tested compounds. However studies have shown that some animal carcinogens despite that they are nongenotoxic (do not react with DNA and they are not mutagenic in short term tests) do induce tumours in specific organs and tissues after a long latency period. The nongenotoxic mechanisms of carcinogenesis are the subject of this book in attempt to answer the question what is the mechanisms and how to predict the risk of such carcinogenic agents.

The volume is devided into seven sections. The first section — promotion — describes the multistage carcinogenesis in the systems of mouse skin, rat hepatocytes and bladder. The relationship of ion deregulation cell injury and neoplasia is discussed as well. The next section is dealing with forced cell proliferation. Using different experimental systems the role of nongenotoxic carcinogens in cell proliferation acting through inbalance in nucleotide pools and cytotoxicity is discussed. Mathematical model was developed to provide some guidance in assessing risk associated with the exposure of humans to nongenotoxic carcinogens. Quantitation of replicative DNA synthesis is proposed as a short term test in detection of nongenotoxic carcinogens. In the next chapter the rodent bioassay is discussed including nongenotoxic mouse liver carcinogenesis, dietary induced cancers and pseudocarcinogenecity in rodent bioassays. Section number four reviewes solid state carcinogenesis. The nongenotoxic nature of tumour induction by foreign body of different origin is described. Examples of nongenotoxic compounds are the subject of the next part of the book. The possible modulation of cell transformation in vitro by a variety of nongenotoxic agents, the role of cell to cell communication in tumour promotion and the genetic and epigenetic mechanisms of presumed nongenotoxic carcinogens in cell culture models are discussed. The last section is giving a critical overview of in vitro tests used to identify potential carcinogens. Finally, the human hazard is discussed and the goal of research to minimize the uncertainity in assesment of human carcinogens is pointed out.

This book should be of interest for oncologists, toxilogists, pharmacologists, environmental scientists and ecologists. Basically, it is deirected to all scientists involved in cancer research who are interested in better understanding of cancer induction.

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