Biochemistry (Moscow), Vol. 68, No. 9, 2003, pp. 1055-1061. Translated from Biokhimiya, Vol. 68, No. 9, 2003, pp. 1287-1294.

Flavonoid Dihydroquercetin, unlike Quercetin, Fails to Inhibit Expression of Heat Shock Proteins under Conditions of Cellular Stress

K. R. Budagova[1], S. V. Zhmaeva[2], A. N. Grigor'ev[2], A. Ya. Goncharova[2], and A. E. Kabakov[1,2]*

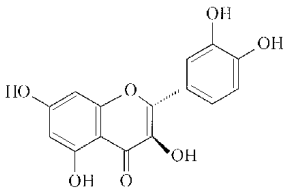
[1]Medical Radiological Research Center, Russian Academy of Medical Sciences, ul. Koroleva 4, Obninsk 249036, Russia; fax: (095) 956-1440; E-mail: mrrc@obninsk.ru
[2]MEDBIOPHARM, Ltd, Inc., ul. Kurchatova 23a, Obninsk 249036, Russia; fax: (08439) 6-8192; E-mail: medbiopharm@medbiopharm.ru

Received January 23, 2003
Revision received March 10, 2003

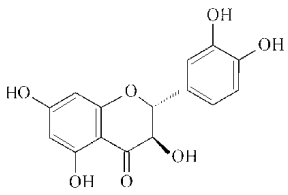
Abstract—Modification by natural flavonoids quercetin and dihydroquercetin of the *in vitro* cell response to hyperthermal and chemical stress was studied. Quercetin completely inhibited the synthesis and intracellular accumulation of 70-kD heat shock protein (HSP70) in response to hyperthermia or to treatment with sodium arsenite, whereas dihydroquercetin in the same or higher doses had no such effect. Stress exposures under conditions of the quercetin-inhibited HSP70 expression significantly increased the percentage of dead and damaged cells compared to the same exposures in the absence of quercetin. On the contrary, dihydroquercetin virtually failed to increase the damage and death of the stress-exposed cells which displayed typical induction of HSP70. The findings suggest a new strategy for pharmacological use of these flavonoids with similar structure.

Key words: bioflavonoids, taxifolin, chaperones, hyperthermia, adaptive response, tolerance

Biologically active polyphenols of the flavonoid family which are present in some products of plant origin have recently attracted sharply increased interest [1, 2]. Most compounds of this family including quercetin and dihydroquercetin (taxifolin)



Quercetin



Dihydroquercetin

are rather strong antioxidants [3-5]. The presence of antioxidant features suggests that these flavonoids could be used as promising artificial scavengers for free radicals which are *in vivo* generated and damage tissues in various pathophysiological processes. Thus, quercetin was shown on animal models to weaken oxidative stress and cell damage in cases of ischemia/reperfusion [6-10], ultraviolet radiation [11], asbestosis [12], and endotoxinemia [13]. In turn, dihydroquercetin had a protective effect as an antioxidant in cerebral ischemia [14], asbestosis [12, 15], experimental hepatitis in rats [16], and also in an *in vitro* model of osteoarthritis [17]. These publications and many others suggest that both flavonoids might be pharmacological agents against oxidative stress on the level of cells and tissues. Because reactive oxygen species are one of the main damaging factors in acute inflammation, ischemia/reperfusion, endotoxinemia, neurodegenerative diseases, diabetes, etc., the therapeutic use of such nontoxic natural antioxidants as quercetin and/or dihydroquercetin seems to be very reasonable and promising, and is now the subject of wide speculation [1-17].

However, quercetin (but not dihydroquercetin) is a well-known inhibitor of expression of heat shock proteins (HSP) in response to hyperthermia and other cellular stresses [18-20]. Excess HSP in the cell can prevent aggregation of unstable cellular proteins under stress conditions and then promote the reactivation or degradation of damaged macromolecules during the recovery period. Thus, induction and accumulation of HSP in the stress-exposed cells is an important adaptive mechanism which minimizes dangerous consequences of a "proteotoxic"

* To whom correspondence should be addressed.

exposure and confers acquisition of resistance to further stresses (for review see [21, 22]). In higher organisms a 70-kD HSP (HSP70) is the main stress-inducible protein, and its overexpression is associated with the cell defense against thermal and oxidative stress [21-23] and also against damage in ischemia/reperfusion [21, 24, 25] and endotoxinemia [26]. Under conditions of *in vitro* cellular stress, quercetin was shown to inhibit both induction of HSP70 and development of tolerance to hyperthermia [20], energy starvation [27, 28], and ultraviolet radiation [29]. The quercetin-inhibited expression of HSP70 in perfused organs after the primary stress increased the cytotoxic effect and functional damage in ischemia/reperfusion [30-33]. An increase in the level of HSP and in radioresistance of bone marrow in mice after whole body hyperthermia was not observed in mice pretreated with quercetin [34]. Thus, on one hand, quercetin, as an antioxidant, can protect tissues under stress conditions [6-11] and, on the other hand, as an inhibitor of HSP induction, suppresses the recovery and adaptive response in the stress-exposed cells [27-34]. The detection of such opposite effects complicates prospects for the use of quercetin in treatment of acute phase conditions which include cellular stress components. But, on the other hand, the inhibition by quercetin of HSP expression seems promising for a combined treatment of malignant tumors because this approach allows us to increase the sensitivity of tumor cells to hyperthermia and chemotherapy [20, 35, 36].

Quercetin-related flavonoids, such as flavone, campferol, genistein, and to a lesser degree luteolin were also found to inhibit the induction of HSP in response to cellular stress [18, 20]. Features of dihydroquercetin were not studied in this context. The purpose of this work was to compare the effects of quercetin and dihydroquercetin on expression of the stress-inducible HSP70 and resistance of the stress-exposed cells. Blood vessels or tumors are usual targets for the therapeutic effect of flavonoids [1, 2, 20, 35, 36]; therefore, this study was performed on vascular endothelial cells and Ehrlich ascites carcinoma (EAC) cells.

MATERIALS AND METHODS

Cell cultures. EAC cells were passaged in nonlinear mice as described in [37]. In *in vitro* experiments EAC cell populations isolated at the logarithmic growth phase were used. The cells were washed and suspended ($6 \cdot 10^6$ cells/ml) in complete Eagle's medium supplemented with 10% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, and antibiotics penicillin (50 units/ml) and streptomycin (100 μg/ml), or in methionine-free Eagle's medium (Flow Laboratories, England).

Endothelial cells were isolated from human umbilical vein and cultured as described in [27] in the RPMI-1640 medium supplemented with 10% fetal calf serum, 20 mM HEPES, 2 mM L-glutamine, 1 mM sodium pyruvate, heparin (100 μg/ml), penicillin (50 units/ml), and streptomycin (100 μg/ml) in gelatinized plastic dishes at 37°C in the presence of 5% CO_2. Monolayer cultures of the first four passages were used in the experiments.

Flavonoids and the treatment of cells. Dihydroquercetin was extracted with water–alcohol mixture from minced larch (*Larix dahurica* T.) wood and purified using the method developed by MEDBIOPHARM (Obninsk, Russia) including distillation, two recrystallizations, and treatment with polyamide (patent pending No. 2002196882 and MPK A61K 35/78). The purity of the resulting preparation was assessed by high-pressure liquid chromatography on a C 18 column (reversed phase) and photometry at $\lambda = 290$ nm (Gilson, France). Moreover, the purity and structural correspondence to the formulae were confirmed by mass-spectrometry. In the experiments a highly purified dihydroquercetin preparation containing no less than 98% of the main substance and quercetin from Serva (Germany) containing 96-98% of the main substance were used. The flavonoid concentrations required for the *in vitro* cell treatment were prepared by dilution in the culture medium of concentrated solutions of flavonoids in dimethylsulfoxide (DMSO). The cells were incubated in the presence of quercetin or dihydroquercetin at 37°C for 1 h before hyperthermia and for 2.5 h before treatment with sodium arsenite and then during both stress exposures. The control samples of cells were supplemented with aliquots of DMSO.

Stress exposures *in vitro*. Cell reactions to thermal or chemical stress exposure were *in vitro* modeled either by heating the samples on a water bath at 44°C for 35 min (hyperthermia) with a subsequent incubation at 37°C, or by treatment with 0.1 mM sodium arsenite ($NaAsO_2$, Fisher Scientific, England) for 1 h with subsequent three washings with the Eagle's medium and incubation at 37°C. In some samples the cells were exposed to stresses in the presence of quercetin or dihydroquercetin in the incubation medium.

Determination of cell damage and death. Specific morphological signs of cell damage (rounding and blebbing of the membranes, pycnosis, etc.) were observed and photographed in phase contrast with an inverted Nikon microscope (Japan). Dead cells were determined by staining with 0.05% Trypan Blue [37]. The cells in contact with the dye were analyzed in a Goryaev's chamber (250-300 cells in the field) and the percent of blue (dead) cells was calculated. Significance of differences in the data was evaluated using Student's *t*-test.

Electrophoresis of proteins and immunoblotting. Cells were lysed in 40 mM Tris-HCl buffer (pH 8.0) supplemented with 10 mM EDTA, 4% SDS, 0.5% dithiothreitol, and 20% glycerol. Before the lysis, this buffer was supplemented with the protease inhibitor phenylmethylsulfonyl fluoride (Serva) (2 μM). Electrophoresis of

lysates was performed in the Laemmli system with a 10% separating polyacrylamide gel as described in [27, 28]. Lysates of $0.5 \cdot 10^6$ cells were added into every well of the concentrating gel.

For immunoblotting, the proteins were transferred from the gel onto a nitrocellulose membrane (0.45 μM; Bio-Rad, USA) using an LKB apparatus (Sweden) at the current of 0.5 mA for 3 h. After the nonspecific binding had been blocked, the membrane was incubated for 1.5 h with monoclonal antibodies specific to the induced form of HSP70 (StressGen, Canada) diluted 1 : 3000 in 25 mM Tris-HCl buffer (pH 7.5) supplemented with 0.5 M NaCl and 0.05% Tween-20. Then the blots washed five times were treated for 1.5 h by peroxidase-conjugated "second" antibodies (StressGen) diluted 1 : 3000. After the blots had been washed following the incubation with the second antibodies, HSP70 bands were developed on an X-ray film by enhanced chemiluminescence using an ECL kit of special reagents (Western blotting analysis system, Amersham, England).

Metabolic labeling of proteins and autoradiography. Immediately after the heating, the EAC cells in the medium with a 96% deficiency of L-methionine were supplemented with D,L-[^{35}S]methionine (produced by the Institute of Physical Energetics, Obninsk) and incubated for 3 h. The concentration of ^{35}S label in the cell-containing medium was 30 μCi/ml. Then the cells were washed five times in the Eagle's medium. The labeled cells were lysed, and electrophoresis of the lysates was performed as described above. The gel dried after electrophoresis was exposed onto a highly sensitive X-ray film (Amersham) in an autoradiography cassette for 24 h at room temperature, then the film was developed to visualize radioactive bands. The 70-kD band corresponding to the induced HSP70 was identified with a set of protein standards (Bio-Rad).

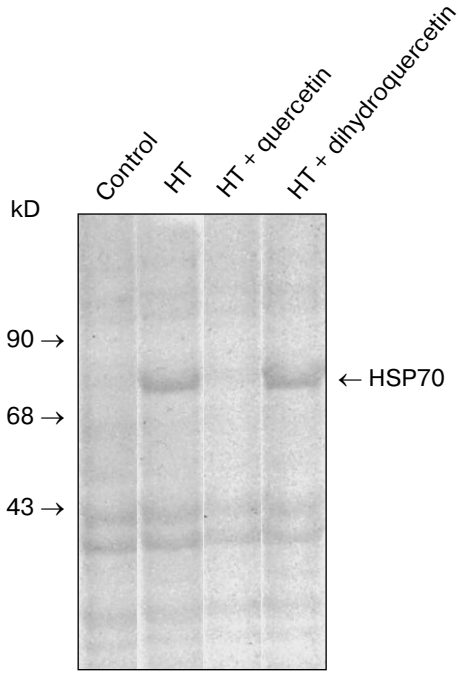


Fig. 1. Effects of quercetin and dihydroquercetin at the concentration of 30 μM on HSP70 synthesis in EAC cells after hyperthermia (HT: 44°C, 35 min). Intensity of the HSP70 synthesis was determined autoradiographically by incorporation of L-[^{35}S]methionine (see "Materials and Methods"). Arrows to the left show positions of molecular weight standards.

RESULTS

In the EAC cells recently exposed to hyperthermal stress, HSP70 was the major product of protein synthesis (Fig. 1). The figure shows that 30 μM quercetin completely inhibited the hyperthermia-stimulated synthesis of HSP70, whereas the same concentration of dihydroquercetin did not change the incorporation of ^{35}S label compared to the control where the cells were heated without pretreatment with the flavonoids (Fig. 1). The synthesis of HSP70 also was not inhibited in the EAC cells heated in the presence of 40-60 μM dihydroquercetin (data not presented).

The effects of quercetin and dihydroquercetin on HSP70 expression in tumor and endothelial cells were determined by immunoblotting with specific antibodies. Figures 2a and 2c show that after the hyperthermal stress contents of HSP70 were strongly increased in both cell types, and in both cases this effect was virtually completely inhibited by 30 μM quercetin. By contrast, dihydroquercetin did not decrease the heating-induced accumulation of HSP70 in the cells of these two cultures (Figs. 2a and 2c).

The treatment of cells with sodium arsenite ($NaAsO_2$) was used as an alternative stress. This reagent modifies SH-groups of cellular proteins and thus somewhat imitates proteotoxic effects of oxidative stress; it is also well known as a "chemical" inducer of HSP [18, 22, 32]. The results are presented in Fig. 2b, and the treatment with sodium arsenite obviously increased threefold the level of HSP70 in EAC cells compared to the control. Similarly to the case of hyperthermia, quercetin suppressed the HSP70 expression in the arsenite-treated EAC cells, whereas dihydroquercetin displayed no such effect (Fig. 2b).

Cytotoxic effects of the thermal and chemical stress on EAC cells, their death after either exposure, were assessed by staining with Trypan Blue (Fig. 3). Four hours after the stress exposure the number of dead cells was insignificantly increased compared to the control in the cells heated in the absence and in the presence of dihydroquercetin (Fig. 3a). The fractions of dead cells were

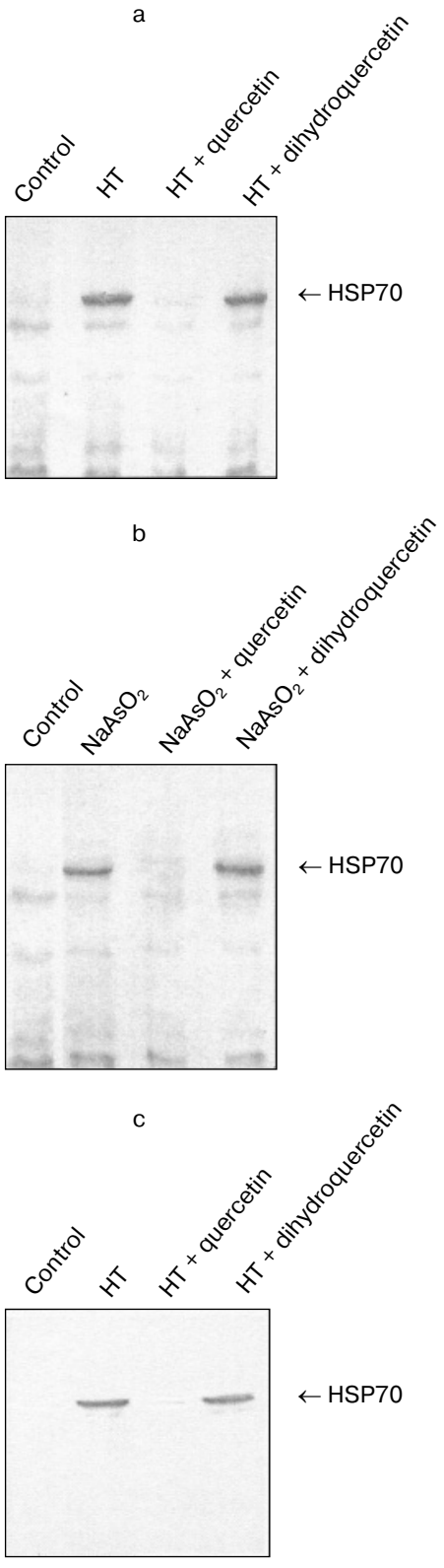


Fig. 2. Effects of quercetin and dihydroquercetin on HSP70 expression induced by thermal stress (HT: 44°C, 35 min) (a, c) and by chemical stress (0.1 mM $NaAsO_2$) in EAC cells (a, b) and in endothelial cells (c). The flavonoid concentration in the incubation medium was 30 μM.

2.74 ± 0.76 (control), 6.03 ± 1.4 (hyperthermia), and 9.6 ± 0.6 (hyperthermia + dihydroquercetin), and the difference in the two latter values is insignificant. However, the number of dead cells in the heated cells with the HSP70 expression inhibited by quercetin was increased about fivefold as compared to the cells heated in the absence of quercetin and was ($30.8 \pm 3.4\%$) ($p < 0.05$). A similar picture was also observed 3 h after termination of the tumor cell contact with arsenite (Fig. 3b). The treatment with arsenite itself killed ($8.03 \pm 1.2\%$) EAC cells, and dihydroquercetin had virtually no effect on this death (the

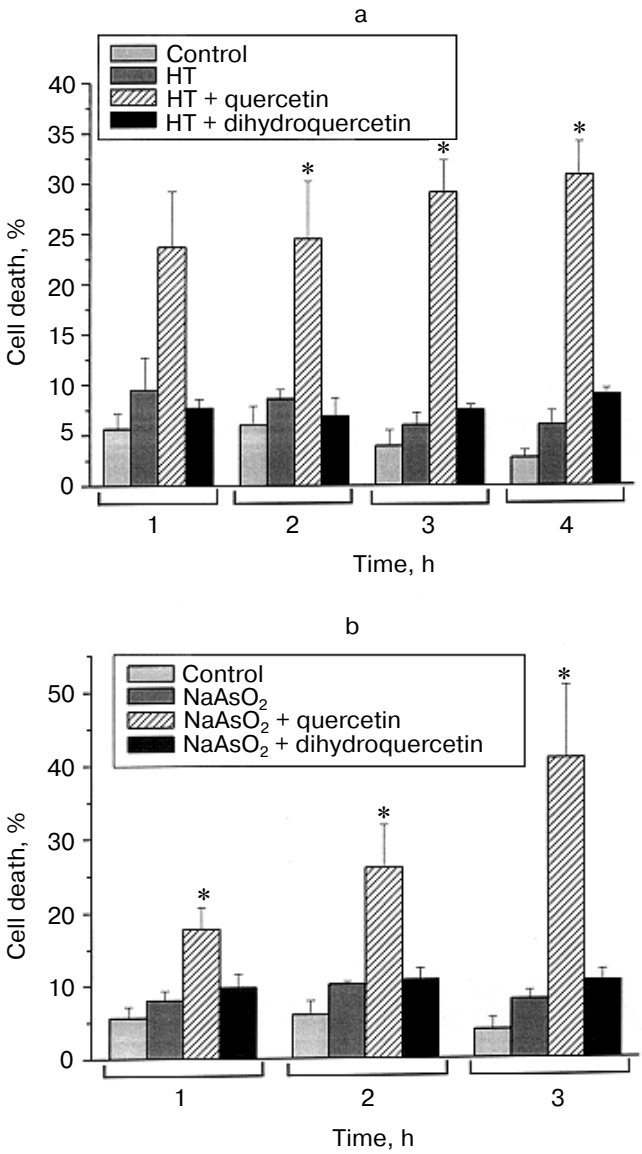


Fig. 3. Effects of quercetin and dihydroquercetin on the death of EAC cells 1-4 h after hyperthermia (HT: 44°C, 35 min) (a) or chemical stress (0.1 mM $NaAsO_2$) (b). * The difference from the control group is significant ($p < 0.05$) by the results of four independent experiments.

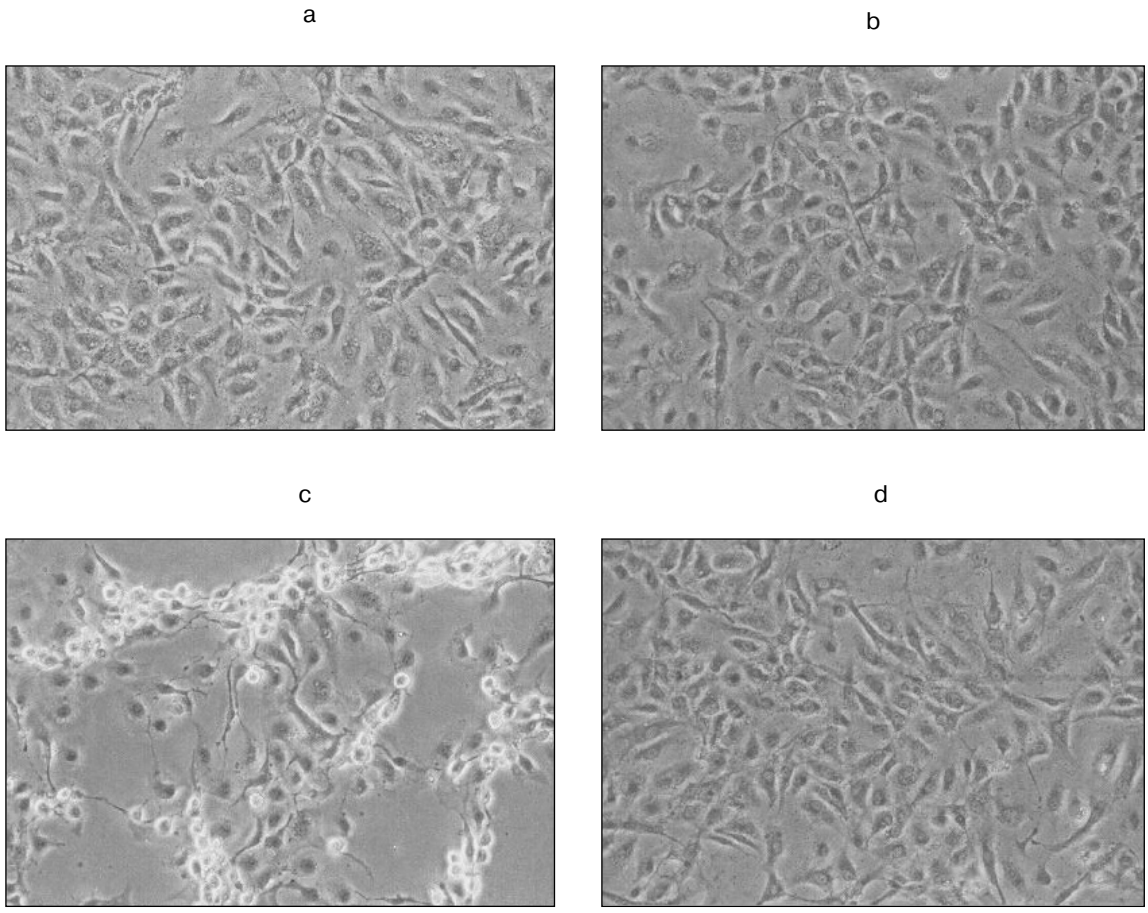


Fig. 4. Morphology of a monolayer culture of endothelial cells 4 h after exposure to hyperthermia (44°C, 35 min). a) Control; b) heated cells; c) heated cells incubated with 30 μM quercetin; d) heated cells incubated with 30 μM dihydroquercetin.

percent of dead cells in the presence of arsenite and dihydroquercetin was 10.5 ± 1.6). By contrast, the treatment with arsenite and quercetin resulted in an about fivefold increase in the cell death when the percent of dead EAC cells was 40.9 ± 9.6 ($p < 0.05$) (Fig. 3b). The difference between the quercetin and dihydroquercetin effects on the EAC cell death clearly correlated with morphological signs of cell damage. While the arsenite + dihydroquercetin combination had almost no effect on the shape of EAC cells, their treatment with arsenite combined with quercetin caused an appearance of a large number (>60%) of cells with specific blebs on the plasma membrane (data not presented) that is considered typical for a strong cytotoxic effect.

Quercetin and dihydroquercetin also had different effects on endothelial cell cultures exposed to hyperthermia (Fig. 4). Thermal stress caused no noticeable morphological changes (Fig. 4b) as compared to the unheated control (Fig. 4a). Cells incubated with 30-60 μM dihydroquercetin also did not differ in morphology from the control specimens (Fig. 4d). These cultures displayed a closely packed monolayer of spread homomorphous cells resembling the morphology of undamaged vascular endothelium. However, the endothelial cell cultures heated with the HSP70 induction inhibited by 30 μM quercetin (Fig. 4b) gradually lost their normal morphology: the cells became rounded, their surface formed blebs, and then the rounded cells stuck into nodules or detached from the substrate (Fig. 4c). Note, that similarly to the experiments with EAC cells, quercetin itself had no noticeable cytotoxic effects; these effects were recorded only with the combination quercetin + stress (hyperthermia).

Thus, the experiments on two different cell types (tumor and normal) and two stresses (thermal and chemical) have shown that dihydroquercetin, unlike quercetin, fails to inhibit the induction of HSP70 in the stress-exposed cells and, consequently, does not decrease their resistance associated with the intracellular accumulation of this protein.

DISCUSSION

Quercetin is known to selectively suppress the transcription of HSP genes, which is mediated by activation of a transcriptional factor HSF1 [18-20]. Obviously, just this property is responsible for the inhibitory effect of quercetin on HSP induction in the stress-exposed cells [27-36]. The findings presented here suggest that unlike quercetin, dihydroquercetin fails to inhibit the expression of the stress-inducible HSP70 (Figs. 1 and 2). It seems likely that dihydroquercetin also does not suppress the expression of other inducible HSP (HSP90, HSP27, etc.), because the transcription of their genes during cellular stress is stimulated by the same mechanism as the transcription of the *HSP70*-gene (for review see [20-22]). Based on the difference in the molecular structures of quercetin and dihydroquercetin, it is suggested that the presence of the double bond between the second and third carbon atoms in the middle (C) ring should be necessary for the inhibitory activity. This conclusion is also supported by the finding that flavone, campferol, genistein, and luteolin also inhibit the induction of HSP [18, 20] and, similarly to quercetin, also have the double bond in this position. Another structural feature common to all these inhibitors is the flavone nucleus itself, and its configuration seems to determine the selectivity of their inhibitory effects only on HSF1-dependent transcription.

The difference found in the biological effects of quercetin and dihydroquercetin seems to be important for prospects of their therapeutic use. Both substances are considered non-toxic antioxidants that can protect organs and tissues in diseases associated with hyperproduction of free radicals [1-17]. But because quercetin suppresses the expression of the stress-inducible HSP and thus decreases the resistance of stress-exposed cells, its use in diseases with a clearly pronounced "stress" component (ischemic insult, endotoxic or septic shock, acute inflammation, etc.) seems unreasonable. Although some authors have recorded a protective (antioxidant) effect of quercetin in models of ischemia/reperfusion [6-10] and endotoxinemia [13], it seems that this defense would be higher in the presence of antioxidant not inhibiting the induction of HSP. Moreover, in those experiments a repeated (or serial) stress was not modeled, and such a stress could be fatal because of the quercetin-caused inhibition of the induction of HSP70 in response to ischemia/reperfusion [30] or endotoxin [38] and, consequently, of abolishment of the tolerance caused by this induction.

Unlike quercetin, dihydroquercetin does not suppress the stress-inducible expression of HSP70 (Figs. 1 and 2); however, dihydroquercetin is a sufficiently strong antioxidant [3-5, 14-17] and its *in vivo* activity is comparable to the antioxidant activity of quercetin [4, 39]. Theoretically, such combination of dihydroquercetin features can ensure an effective defense of cells and tissues in acute phase situations accompanied by oxidative stress when quercetin is likely to induce damage by suppressing the induction of HSP. Such situations occur, for example, in acute local inflammation (such as arthritis), endotoxinemia and sepsis, acute ischemia and its consequences, some traumas, burns, and organ dysfunctions, and also reperfusion stress which is often associated with surgical operations. Note that promising results have already been obtained with dihydroquercetin on *in vitro* and animal models of certain pathological states [14-17].

However, an artificial suppression by quercetin of the expression of inducible HSP is also interesting for practice, namely, as an approach to increase the efficiency of combined oncotherapy. Many tumors *in vivo* increase HSP expression spontaneously or after treatment and, as a result, acquire resistance to hyperthermia and some drugs of chemotherapy. Sometimes tumor cell transition into a tolerant phenotype can be prevented with quercetin [20, 35]. In the same respect, it is important that quercetin also inhibits the transcription of the *MDR1*-gene [20, 36] which is responsible for the "multiple drug resistance" of aggressive tumors unresponsive to chemotherapy. The transcription of the *MDR1*-gene, similarly to the transcription of *HSP*-genes, is initiated by the activated HSF1 and, therefore, is also sensitive to quercetin [20, 36].

The findings and reasoning presented here should be taken into consideration for the development of approaches to the use of flavonoids in medicine.

The work was supported by INTAS (the project No. 01-0409). The authors are grateful to A. L. Bryantsev for his help in photography of the cells.

REFERENCES

1. Tjukavkina, N. A., Rulenko, I. A., and Kolesnik, Yu. A. (1996) *Vopr. Pitaniya*, **2**, 33-38.
2. Pietta, P. (1998) in *Flavonoids in Health and Disease* (Rice-Evance, C. A., and Packer, L., eds.) Marcel Dekker, Inc., New York, pp. 61-110.
3. Bors, W., Heller, W., Michel, C., and Saran, M. (1990) *Meth. Enzymol.*, **186**, 343-355.
4. Areias, F. M., Rego, A. C., Oliveira, C. R., and Seabra, R. M. (2001) *Biochem. Pharmacol.*, **62**, 111-118.
5. Ishige, K., Schubert, D., and Sagara, Y. (2001) *Free Rad. Biol. Med.*, **30**, 433-446.
6. Kolchin, Yu. N., Maksyutina, N. P., Balanda, P. P., Luik, A. I., Bulakh, V. N., and Moibenko, A. A. (1991) *Farmakol. Toksikol.*, **54**, 20-23.
7. Huk, I., Brovkovych, V., Vili, J. N., Weigel, G., Neumayer, Ch., Partika, L., Patton, S., and Malinski, T. (1998) *Br. J. Surg.*, **85**, 1080-1085.
8. Mojzis, J., Hviscova, K., Germanova, D., Bukovicova, D., and Mirossay, L. (2001) *Physiol. Res.*, **50**, 501-506.
9. Brookes, P. S., Digerness, S. B., Parks, D. A., and Darley-Usmar, V. (2002) *Free Rad. Biol. Med.*, **32**, 1220-1228.

10. Inal, M., Altinisik, M., and Bilgin, M. D. (2002) *Cell Biochem. Funct.*, **20**, 291-296.
11. Kahraman, A., and Inal, M. E. (2002) *J. Appl. Toxicol.*, **22**, 303-309.
12. Kostyuk, V. A., and Potapovich, A. I. (1998) *Arch. Biochem. Biophys.*, **355**, 43-48.
13. Abd El-Gawad, H. M., and Khalifa, A. E. (2001) *Pharmacol. Res.*, **43**, 257-263.
14. Plotnikov, M. B., Logvinov, S. V., Pugachenko, N. V., Maslov, M. Yu., Aliev, O. I., Tjukavkina, N. A., Suslov, N. I., and Potapov, A. V. (2000) *Byull. Eksp. Biol. Med.*, **130**, 1080-1083.
15. Kostyuk, V. A., Potapovich, A. I., Vladykovskaya, E. N., Korkina, L. G., and Afanas'ev, I. B. (2001) *Arch. Biochem. Biophys.*, **385**, 129-137.
16. Teselkin, Yu. O., Babenkova, I. V., Kolhir, V. K., Baginskaya, A. I., Tjukavkina, N. A., Kolesnik, Yu. A., Selivanova, I. A., and Eichholz, A. A. (2000) *Phytother. Res.*, **14**, 160-162.
17. Jovanovic, D. V., Mineau, F., Notoya, K., Reboul, P., Martel-Pelletier, J., and Pelle, J. P. (2002) *J. Rheumatol.*, **29**, 2165-2175.
18. Hosokawa, N., Hirayoshi, K., Nakai, A., Hosokawa, Y., Marui, N., Yoshida, M., Sakai, T., Nishino, H., Aoike, A., Kawai, K., and Nagata, K. (1990) *Cell Struct. Funct.*, **15**, 393-401.
19. Hosokawa, N., Hirayoshi, K., Kudo, H., Takeshi, H., Aoike, A., Kawai, K., and Nagata, K. (1992) *Mol. Cell. Biol.*, **12**, 3490-3498.
20. Nagata, K. (1996) in *Stress-Inducible Cellular Response* (Feige, U., Morimoto, R. I., Yahara, I., and Polla, B. S., eds.) Birkhauser Verlag, Basel, pp. 467-481.
21. Kabakov, A. E., and Gabai, V. L. (1997) *Heat Shock Proteins and Cytoprotection: ATP-Deprived Mammalian Cells*, Landes Company, Austin.
22. De Maio, A. (1999) *Shock*, **11**, 1-12.
23. Chong, K. Y., Lai, C. C., Lille, S., Chang, C., and Su, C. Y. (1998) *J. Mol. Cell. Cardiol.*, **30**, 599-608.
24. Suzuki, K., Sawa, Y., Kaneda, Y., Ichikawa, H., Shirakura, T., and Matsuda, H. (1997) *J. Clin. Invest.*, **99**, 1645-1650.
25. Jayakumar, J., Suzuki, K., Sammut, I. A., Smolenski, R. T., Khan, M., Latif, N., Abunasra, H., Murtuza, B., Amrani, M., and Yacoub, M. H. (2001) *Circulation*, **104**, I303-I307.
26. Lau, S. S., Griffin, T. M., and Mestril, R. (2000) *Am. J. Physiol.*, **278**, H1439-H1445.
27. Loktionova, S. A., Ilyinskaya, O. P., and Kabakov, A. E. (1998) *Am. J. Physiol.*, **275**, H2147-H2158.
28. Kabakov, A. E., Budagova, K. R., Latchman, D. S., and Kampinga, H. H. (2002) *Am. J. Physiol.*, **283**, C521-C534.
29. Trautinger, F., Kindas-Mugge, I., Barlan, B., Neuner, P., and Knobler, R. M. (1995) *J. Invest. Dermatol.*, **105**, 160-162.
30. Nakata, N., Kato, H., and Kogure, K. (1993) *Neuroreport*, **4**, 695-698.
31. Pshennikova, M. G., Prodius, P. A., Sazontova, T. G., Golantsova, N. E., and Malyshev, I. Yu. (1998) *Sechenov Ros. Fiziol. Zh.*, **84**, 1214-1222.
32. Tsuruma, T., Yagihashi, A., Watanabe, N., Yajima, T., Kameshima, H., Araya, J., and Hirata, K. (1999) *Surgery*, **125**, 385-395.
33. Kelly, K. J., Baird, N. R., and Greene, A. L. (2001) *Kidney Int.*, **59**, 1798-1802.
34. Malyutina, Ya. V., Semina, O. V., Semenets, T. N., Budagova, K. R., Shinkarkina, A. P., Kabakov, A. E., and Poverennyi, A. M. (2001) *Radiats. Biol. Radioekol.*, **41**, 167-170.
35. Asea, A., Ara, G., Teicher, B. A., Stevenson, M. A., and Calterwood, S. K. (2001) *Int. J. Hypertherm.*, **17**, 347-356.
36. Kim, S. H., Yeo, G. S., Lim, Y. S., Kang, C. D., Kim, C. M., and Chung, B. S. (1998) *Exp. Mol. Med.*, **30**, 87-92.
37. Gabai, V. L., Kabakov, A. E., Makarova, Yu. M., Mosina, V. A., and Mosin, A. F. (1994) *Biochemistry (Moscow)*, **59**, 399-404.
38. Zhang, Y. H., Takahashi, K., Jiang, G. Z., Zhang, X. M., Kawai, M., Fukada, M., and Yokochi, T. (1994) *Infect. Immun.*, **62**, 4140-4144.
39. Silva, M. M., Santos, M. R., Caroco, G., Rocha, R., Justino, G., and Mira, L. (2002) *Free Rad. Res.*, **36**, 1219-1227.