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Original Article

3', 4'-Dihydroxyflavone enhances all-*trans* retinoic acid-induced superoxide-generating activity through up-regulating transcription of gp91-phox in human monoblastic U937 cells, as opposed to flavone and other hydroxyflavone derivatives

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ABSTRACT — Flavones are belonging to flavonoids group and show diverse biological functions. Therefore, they have much attention as drugs for maintaining human health via contributing prevention and treatment of various diseases like cancers, diabetes, neurodegenerative diseases, ischemic stroke, inflammation diseases and cardiovascular diseases. On the other hand, human monoblastic leukemia U937 cells have been used as an excellent *in vitro* model system for macrophage development induced in response to various reagents such as all-*trans* retinoic acid (RA). Here, we investigated the effects of flavones (flavone and its hydroxy derivatives) on the RA-induced O_2^--generating activity of U937 cells. Very interestingly, at a concentration of 20 μM, 3', 4'-dihydroxyflavone caused up-regulation of the RA-induced O_2^--generating activity (to ~ 170%) although flavone and other hydroxyflavone derivatives tested showed remarkable inhibitory effects on the RA-induced O_2^--generating activity. The promoting effects of 3', 4'-dihydroxyflavone on the RA-induced O_2^--generating activity showed the maximum value at a concentration of 10 μM. Semiquantitative RT-PCR and immunoblotting revealed that 10 μM 3', 4'-dihydroxyflavone up-regulates the RA-induced O_2^--generating activity via enhancing gene expression of gp91-phox (mRNA level: to ~ 160%, protein level: to ~ 200%) while 10 μM 5, 7-dihydroxyflavone and 10 μM 3', 4', 5, 7-tetrahydroxyflavone down-regulate the RA-induced O_2^--generating activity via inhibiting gene expression of gp91-phox and p47-phox. These findings also showed that there may be various risks involved in use of phytochemical mixtures.

Key words: Flavone, Hydroxyflavone, Superoxide, Macrophage, All-*trans* retinoic acid

INTRODUCTION

Phytochemicals are secondary metabolites in higher plants, and protect them from various risks, e.g., UVB irradiation, environmental toxicants and numerous microbial infections. They found abundantly in many kinds of vegetable food and also show diverse biological functions. Therefore, many phytochemicals have attracted

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attention as alternate drugs for maintaining human health. Polyphenols, one of the major groups of phytochemicals, can be classified into several categories such as flavonoids, stilbenoids and phenolic acids (Arora *et al*., 2019). These compounds play important roles in prevention and treatment of various diseases, like, cancers, diabetes, neurodegenerative diseases, ischemic stroke, inflammation diseases, cardiovascular diseases, and so on (Braakhuis *et al*., 2016; Zhou *et al*., 2016; Arora *et al*., 2019; Miyata *et al*., 2019; Leri *et al*., 2020).

We have studied the effects of various phytochemicals on the all-*trans* retinoic acid (RA)-induced superoxide anion (O_2^-)-generating activity of human monoblast U937 cells such as curcumin (Kikuchi *et al*., 2010), resveratrol (Kikuchi *et al*., 2018), chalcones (Kikuchi *et al*., 2019), sulforaphane (Akiyoshi *et al.*, 2019), ellagic acid and urolithin A (Kikuchi *et al*., 2021). As is well known, in response to various agents such as RA, U937 cells differentiate to macrophage-like cells which can produce O_2^- (Kikuchi *et al*., 1994). The O_2^--generating system in leukocytes needs five specific protein factors (the small [p22-phox] and the large [gp91-phox] subunits of cytochrome b_{558} heterodimer in membranes, cytosolic p40-phox, p47-phox and p67-phox) and small G-protein Rac (Dagher and Pick, 2007; Panday *et al*., 2015). Here, we focused on flavones, and investigated their influences on the RA-induced O_2^--generating activity of U937 cells. Flavones are belonging to flavonoids which are subdivided into flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanidins (Arora *et al*., 2019), and show also numerous pharmacological activities against various diseases (Nabavi *et al*., 2015; Theoharides *et al*., 2015; Liu *et al*., 2016; Hostetler *et al*., 2017; Liang *et al*., 2017; Yan *et al*., 2017; Farkhondeh *et al*., 2019; Saraei *et al*., 2019; Moghadam *et al*., 2020). Regarding human neutrophils and mononuclear blood cells, Davalos *et al*. (2009) reported that red grape juice polyphenols (mixture of various polyphenols) reduce the O_2^--generating activity via inhibition of gene expression of p22-phox, gp91-phox and p47-phox. However, the understanding of the effects of individual flavones against the RA-induced O_2^--generating activity of U937 cells is still poor.

In this paper, we investigated the effects of flavones (flavone and its hydroxy derivatives) on the RA-induced O_2^--generating activity of U937 cells, and revealed that 3', 4'-Dihydroxyflavone enhances the RA-induced O_2^--generating activity through up-regulating transcription of gp91-phox, as opposed to flavone and other hydroxyflavone derivatives tested.

MATERIALS AND METHODS

Materials

3'-Hydroxyflavone, 4'-hydroxyflavone (Funakoshi, Tokyo, Japan), flavone, 5-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone, 5, 7-dihydroxyflavone (chrysin), 7, 8-dihydroxyflavone, 3', 4'-dihydroxyflavone, 5, 6, 7-trihydroxyflavone (baicalein), 3', 4', 5, 7-tetrahydroxyflavone (luteolin) (Tokyo Chemical Industry, Tokyo, Japan), 4',5,7-trihydroxyflavone (apigenin) (Nacalai Tesque, Kyoto, Japan), RPMI-1640 culture medium (Gibco Laboratories, Gaithersburg, MD, USA), phorbol 12-myristate 13-acetate (PMA), RA (Sigma, St Louis, MO, USA), fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS, USA), plasmocin (InvivoGen, San Diego, CA, USA), monoclonal anti-gp91-phox antibody, monoclonal anti-p47-phox antibody (BD Biosciences, San Jose, CA, USA), monoclonal anti-p67-phox antibody, horseradish peroxidase-conjugated anti-goat immunoglobulin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p40-phox antibody (GeneTex, Irvine, CA, USA), monoclonal anti-β-actin antibody, monoclonal anti-Na^+/K^+-ATPase antibody (Abcam, Cambridge, UK), and horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin (Promega, Madison, WI, USA) were obtained from companies indicated respectively. Monoclonal anti-human p22-phox antibody (449) was kindly provided by Dr. Roos and Dr. Verhoeven (Sanquin Research, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, The Netherlands).

Cell culture and treatment with flavone and its hydroxy derivatives

Human monoblastic leukemia U937 cells (RCB0435) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were grown in RPMI-1640 culture medium containing 10% FBS and 5 μg/mL plasmocin as described (Kikuchi *et al*., 2019, 2021). Cells (1.0×10^6) in 5 mL of culture medium were incubated with any of flavone and its hydroxy derivatives (10 or 20 μM) in the presence of 1 μM RA for 48 hr. In addition, regarding 3',4'-dihydroxyflavone, cells were also incubated with 5 or 15 μM 3',4'-dihydroxyflavone in the presence of 1 μM RA at 37°C for 48 hr.

Measurement of O_2^- generation

Measurement of O_2^- generation was performed by Lumat^3 LB9508 luminometer (Berthold Technologies, Bad Wildbad, Germany) using luminol and Diogenes-

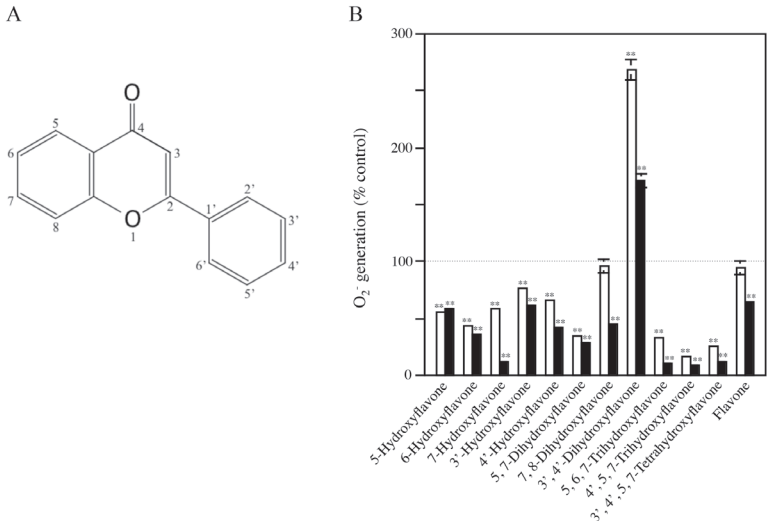

Fig. 1. Effects of flavone and its hydroxy derivatives on the RA-induced O_2^--generating activity of U937 cells. (A) Structural and numerical representations of the flavone skeleton. (B) After cultivation with RA for 48 hr in the absence or presence of flavones (10 μM [open bars] or 20 μM [closed bars]), cells (1 x 10^5 cells/mL) were stimulated with 200 ng/mL PMA at 37°C. PMA-induced chemiluminescence was measured at 10 min after stimulation using a Lumat^3 LB9508 luminometer as described previously (Kikuchi *et al*., 2018). Quantitative data of O_2^- generation are indicated as percentages of the control value obtained from RA-treated U937 cells. Data represent the averages of three separate experiments; error bars indicate standard deviation. **, $p < 0.01$ compared with the data of RA-treated U937 cells.

luminol chemiluminescence probes as described previously (Kikuchi *et al*., 2018).

Semiquantitative RT-PCR

Cells (2.0 x 10^6) in 5 mL of the culture medium were incubated with 1 μM RA in the absence or presence of 10 μM flavones (flavone, 5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone or 3', 4', 5', 7-tetrahydroxyflavone) at 37°C for 48 hr. Total RNA was isolated from the cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA). RT reaction was performed with a first strand cDNA synthesis kit ReverTra Ace-α (Toyobo, Osaka, Japan). Semiquantitative RT-PCR was carried out as described previously (Kikuchi *et al*., 2011, 2021). Sequence data of primers were also described (Kikuchi *et al*., 2011, 2021). Human GAPDH gene was used as internal controls. Semiquantitative RT-PCR data were obtained using a luminescent image analyzer STAGE-5100 (AMZ System Science, Osaka, Japan), analyzed by Quant-AMZ software (TotalLab., Newcastle upon Tyne, UK) as described (Kikuchi *et al*., 2021).

Immunoblot analysis

Cells (2.0 x 10^6) in 5 mL of the culture medium were incubated with 1 μM RA in the absence or presence of 10 μM flavones (flavone, 5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone or 3', 4', 5', 7-tetrahydroxyflavone) at 37°C for 48 hr, disrupted in 100 μL of 50 mM Tris-HCl buffer (pH 7.5) containing 0.25 M sucrose, 2 mM EDTA and 1 mM PMSF, and divided into cytosolic fractions and membrane fractions by centrifugation. These protein samples were subjected to SDS-PAGE followed by immunoblot analysis as described previously. Data analyses were carried out using a luminescent image analyzer STAGE-5100. Human β-actin (for cytosolic fractions) and Na^+/K^+-ATPase (for membrane fractions) were used as controls (Kikuchi *et al*., 2019, 2021).

Statistical analysis

Quantitative data are presented as averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

RESULTS AND DISCUSSION

First, in order to know the influences of flavone and various hydroxyflavone derivatives (Fig. 1A) on the RA-induced O_2^--generating activity of U937 cells, the cells were treated with 10 μM or 20 μM of each reagent in the presence of 1 μM RA. As shown in Fig. 1B, at a final concentration of 20 μM, flavone and most of hydroxy-

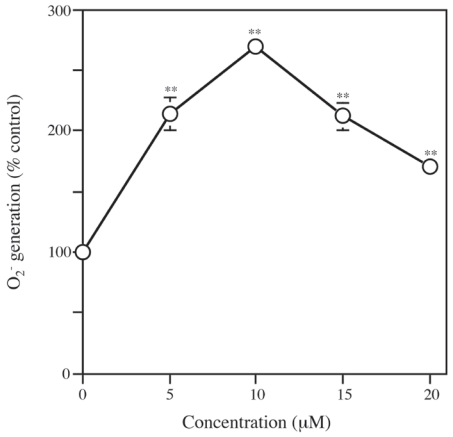

Fig. 2. Dose-dependent effects of 3', 4'-dihydroxyflavone on the RA-induced O_2^--generating activity of U937 cells. After cultivation with RA for 48 hr in the absence or presence of 3', 4'-dihydroxyflavone (5, 10, 15 and 20 μM), cells (1 x 10^5 cells/mL) were stimulated with 200 ng/mL PMA at 37°C. PMA-induced chemiluminescence was measured at 10 min after stimulation using a Lumat^3 LB9508 luminometer as described previously (Kikuchi *et al*., 2018). Quantitative data of O_2^- generation are indicated as percentages of the control value obtained from RA-treated U937 cells. Data represent the averages of three separate experiments; error bars indicate standard deviation. **, $p < 0.01$ compared with the data of RA-treated (without 3', 4'-dihydroxyflavone) U937 cells.

flavone derivatives tested showed remarkable inhibitory effects on the O_2^--generating activity of U937 cells in the presence of RA. In contrast, very interestingly, only 3', 4'-dihydroxyflavone brought about markedly up-regulated the O_2^--generating activity of U937 cells (10 μM: to ~ 270%, 20 μM: to ~ 170%) in the presence of RA (Fig. 1B). As a side note, flavones used in this study showed no effect on the viability of U937 cells up to 20 μM even in the presence of RA (data not shown). In addition, the promoting effects of 3', 4'-dihydroxyflavone on the RA-induced O_2^--generating activity showed the maximum value at a concentration of 10 μM (Fig. 2). The cause of the O_2^--generation reducing effects at over 10 μM is still unknown. These results suggested that positions and numbers of hydroxy groups in hydroxyflavone derivatives may participate in their effects on the RA-induced O_2^--generating activity of U937 cells.

Second, to investigate the effects of flavone and three hydroxyflavone derivatives (5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone) on transcription levels of five genes essential for the O_2^--generating activity (p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox) in detail, we performed semiquantitative RT-PCR analysis (Fig. 3). The experiments were performed using these flavones at a final concentration of 10 μM because the promoting effects of 3', 4'-dihydroxyflavone on the RA-induced O_2^--generating activity showed the maximum value at a concentration of 10 μM (see Fig. 2). As shown in Fig. 1B, at a final concentration of 10 μM, flavone showed no effect on the RA-induced O_2^--generating activity, 5, 7-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone decreased the RA-induced O_2^--generating activity, and 3', 4'-dihydroxyflavone increased the RA-induced O_2^--generating activity. Total RNAs were prepared from RA-treated, RA plus 10 μM flavone-treated, RA plus 10 μM 5, 7-dihydroxyflavone-treated, RA plus 10 μM 3', 4'-dihydroxyflavone-treated, and RA plus 10 μM 3', 4', 5, 7-tetrahydroxyflavone-treated U937 cells. Quantitative data were indicated as percentages of control values obtained from RA-treated U937 cells. Flavone showed no effect on transcription levels of these five genes. As expected, transcription levels of gp91-phox were up-regulated in RA plus 3', 4'-dihydroxyflavone-treated U937 cells (to ~ 160%). In contrast, transcription levels of gp91-phox were certainly down-regulated in the cells treated with RA plus 5, 7-dihydroxyflavone (to ~ 60%) or 3', 4', 5, 7-tetrahydroxyflavone (to ~ 65%). In addition, transcription levels of p47-phox were also markedly down-regulated in the cells treated with RA plus 5, 7-dihydroxyflavone (to ~ 60%) or 3', 4', 5, 7-tetrahydroxyflavone (to ~ 40%). These results showed that 5, 7-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone attenuate transcription of gp91-phox and p47-phox, whereas 3', 4'-dihydroxyflavone enhances transcription of gp91-phox.

Finally, to examine the influences flavone and three hydroxyflavone derivatives (5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone) on amounts of five proteins essential for the O_2^--generating system (p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox), immunoblot assay was carried out using antibody specific for each protein (Fig. 4A). Quantitative data were indicated as percentages of control values obtained from RA-treated U937 cells (Fig. 4B). Protein levels of p22-phox (to ~ 180%) and gp91-phox (to ~ 200%) were obviously up-regulated in RA plus 3', 4'-dihydroxyflavone-treated U937 cells. In contrast, protein levels of p22-phox and gp91-phox were down-regulated in the cells treated with RA plus 5, 7-dihydroxyflavone (p22-phox: to ~ 80%, gp91-phox: to ~ 50%) or 3', 4', 5, 7-tetrahydroxyflavone (p22-phox: to ~ 80%, gp91-phox: ~ 60%). Although all flavones tested showed

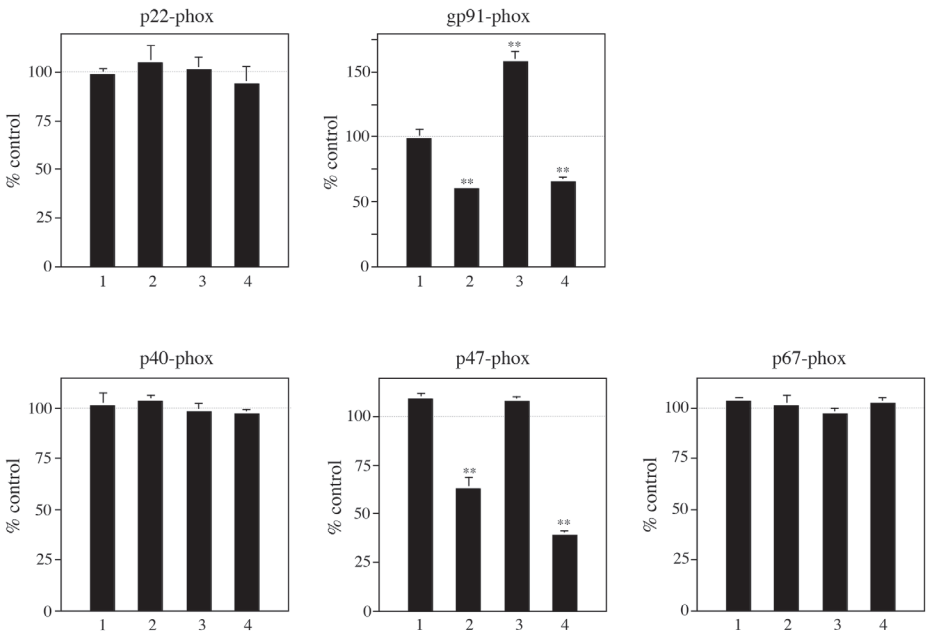

Fig. 3. Effects of flavones (flavone, 5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone) on transcription of the O_2^--generating system-related factors. The mRNA levels of p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox were determined by semiquantitative RT-PCR using total RNA extracted from RA-treated, RA plus 10 μM flavone-treated (lane 1), RA plus 10 μM 5, 7-dihydroxyflavone-treated (lane 2), 10 μM 3', 4'-dihydroxyflavone-treated (lane 3) and 10 μM 3', 4', 5, 7-tetrahydroxyflavone-treated (lane 4) U937 cells as described (Kikuchi *et al*., 2021). PCR data before reaching the plateau were analyzed by Quant-AMZ software using STAGE-5100 image analyzer. Data calibrated with the internal controls (human GAPDH gene) are indicated as percentages of control values obtained from RA-treated U937 cells, and represent the average of three separate experiments. Statistical differences were calculated using Student's *t* test. Error bars indicate standard deviation. **, $p < 0.01$ compared with the data of RA-treated cells.

no effect on transcription levels of p22-phox (see Fig. 3), three hydroxyflavone derivatives (5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone) altered protein levels of p22-phox. As mentioned above, p22-phox and gp91-phox proteins cooperatively assemble to form cytochrome b_{558} heterodimer in the plasma membrane (Dagher and Pick, 2007; Panday *et al*., 2015). Our previous study showed that expression level of gp91-phox protein limits the O_2^--generating activity of U937 cells (Kikuchi *et al*., 1994). In addition, as shown in our previous reports (Kikuchi *et al*., 2018, 2021), it is believed that amounts of p22-phox protein may tend to depend on that of gp91-phox in U937 cells. On the other hand, reflecting the transcription levels of p47-phox, its protein levels were certainly down-regulated in the cells treated with RA plus 5, 7-dihydroxyflavone (to ~ 60%) or 3', 4', 5, 7-tetrahydroxyflavone (to ~ 60%).

Taken together, our data obtained in this study revealed that 3', 4'-dihydroxyflavone up-regulates the RA-induced O_2^--generating activity of U937 cells via enhancing gene expression of gp91-phox while 5, 7-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone down-regulate the RA-induced O_2^--generating activity of U937 cells through inhibiting gene expression of gp91-phox and p47-phox. These results suggested that dihydroxylation of flavone at the 5- and 7-positions may cause down-regulation of gene expression of gp91-phox and p47-phox, dihydroxylation of flavone at the 3'- and 4'-positions may cause up-regulation of gp91-phox gene expression, and dihydroxylation of flavone at the 5- and 7-positions may cancel the effect of dihydroxylation of flavone at the 3'- and 4'-positions on gp91-phox gene expression. Significantly, dihydroxylation of flavone at the 3'- and 4'-positions enhances the RA-induced O_2^--generating activity of U937 cells although monohydroxylation of flavone at the 3'- or 4'-positions attenuates it (see Fig. 1B). This problem

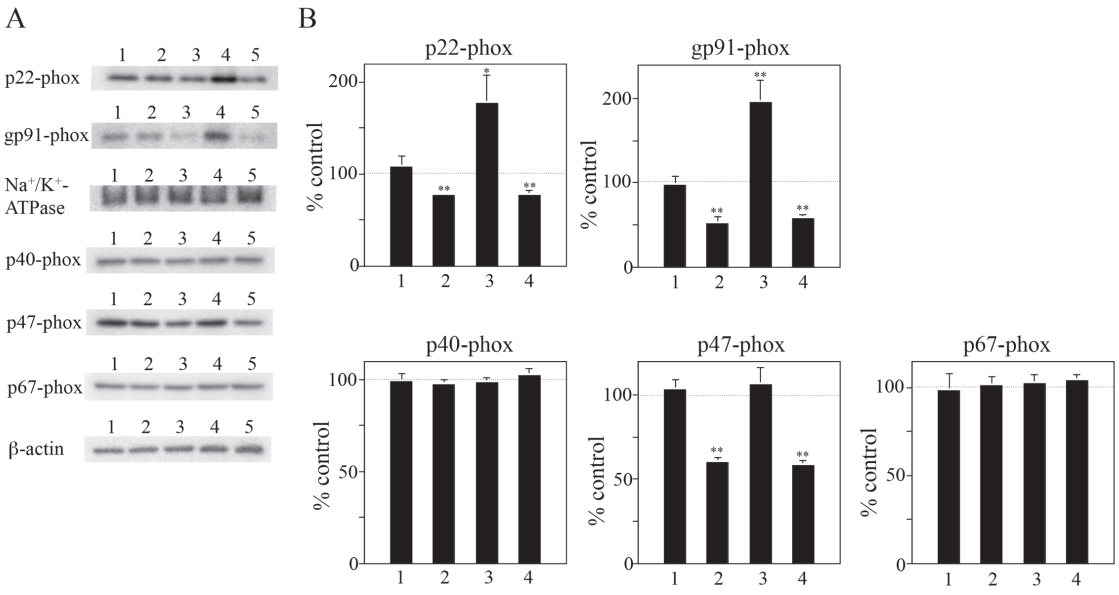

Fig. 4. Effects of flavones (flavone, 5, 7-dihydroxyflavone, 3', 4'-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone) on protein levels of the five O_2^--generating system-related factors. (A) Typical immunoblot profiles. Membrane (for p22-phox and gp91-phox) and cytosolic (for p40-phox, p47-phox and p67-phox) fractions were prepared from RA-treated (lane 1), RA plus 10 μM flavone-treated (lane 2), RA plus 10 μM 5, 7-dihydroxyflavone-treated (lane 3), 10 μM 3', 4'-dihydroxyflavone-treated (lane 4) and 10 μM 3', 4', 5, 7-tetrahydroxyflavone-treated (lane 5) U937 cells, and protein levels of the five O_2^--generating system-related factors were determined by immunoblot analysis. Human Na^+/K^+-ATPase (for membrane fractions) and β-actin (for cytosolic fractions) were used as controls. (B) Quantitative data of immunoblot analysis. Data of RA plus 10 μM flavone-treated (lane 1), RA plus 10 μM 5, 7-dihydroxyflavone-treated (lane 2), 10 μM 3', 4'-dihydroxyflavone-treated (lane 3) and 10 μM 3', 4', 5, 7-tetrahydroxyflavone-treated (lane 4) U937 cells are indicated as percentages of control value obtained from RA-treated U937 cells, and represent the average of three separate experiments. Statistical differences were calculated using Student's *t* test. Error bars indicate standard deviation. *, $p < 0.05$, **, $p < 0.01$ compared with the data of RA-treated cells.

concerning positions and numbers of hydroxy groups in hydroxyflavone derivatives remains to be solved. These findings also showed the possibility that 3', 4'-dihydroxyflavone can be available for an effective activator of phagocytes, and 5, 7-dihydroxyflavone and 3', 4', 5, 7-tetrahydroxyflavone may be used as agents against various reactive oxygen species-mediated diseases such as inflammatory diseases. Our results in this paper may promote effective utilization of 3', 4'-dihydroxyflavone although there is not much report concerning the bioactivities of 3', 4'-dihydroxyflavone (Schlupper *et al*., 2006; Kim *et al*., 2018). In addition, it is worthy of notice that similar chemical compounds can show the opposite effects on the same biological reactions. Phytochemicals often use as mixtures such as crude extract of plants. Our results showed that there may be various risks involved in treatment with phytochemicals as mixtures; for example, 5, 7-dihydroxyflavone attenuates the O_2^--generating activity of leukocytes, contamination by 3', 4'-dihydroxyflavone may interfere the activity of 5, 7-dihydroxyflavone. Moreover, it is possible that mixtures of various phytochemical may show unexpected toxicity. Therefore, studies on bioactivities and clinical applications of phytochemicals must be carried out using pure compounds.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Akiyoshi, S., Kikuchi, H., Kuribayashi, F. and Madhyastha, H. (2019): Sulforaphane displays the growth inhibition, cytotoxicity and enhancement of retinoic acid-induced superoxide-generating activity in human monoblastic U937 cells. Fundam. Toxicol. Sci., **6**, 319-325.

Arora, I., Sharma, M. and Tollefsbol, T.O. (2019): Combinatorial epigenetics impact of polyphenols and phytochemicals in cancer prevention and therapy. Int. J. Mol. Sci., **20**, 4567.

Braakhuis, A.J., Campion, P. and Bishop, K.S. (2016): Reducing breast cancer recurrence: the role of dietary polyphenolics. Nutrients, **8**, 547.

Dagher, M.C. and Pick, E. (2007): Opening the black box: lessons from cell-free systems on the phagocyte NADPH-oxidase. Biochimie, **89**, 1123-1132.

Davalos, A., de la Pena, G., Sanchez-Martin, C.C., Guerra, M.T., Bartolome, B. and Lasuncion, M.A. (2009): Effects of red grape juice polyphenols in NADPH oxidase subunit expression in human neutrophils and mononuclear blood cells. Br. J. Nutr., **102**, 1125-1135.

Farkhondeh, T., Samarghandian, S. and Bafandeh, F. (2019): The cardiovascular protective effects of chrysin: a narrative review on experimental researches. Cardiovasc. Hematol. Agents Med. Chem., **17**, 17-27.

Hostetler, G.L., Ralston, R.A. and Schwartz, S.J. (2017): Flavones: food sources, bioavailability, metabolism, and bioactivity. Adv. Nutr., **8**, 423-435.

Kikuchi, H., Fujinawa, T., Kuribayashi, F., Nakanishi, A., Imajoh-Ohmi, S., Goto, M. and Kanegasaki, S. (1994): J. Biochem., **116**, 742-746.

Kikuchi, H., Kuribayashi, F., Kiwaki, N. and Nakayama, T. (2010): Curcumin dramatically enhances retinoic acod-induced superoxide generating activity via accumulation of p47-phox and p67-phox proteins in U937 cells. Biochem. Biophys. Res. Commun., **395**, 61-65.

Kikuchi, H., Kuribayashi, F., Kiwaki, N., Takami, Y. and Nakayama, T. (2011): GCN5 regulates the superoxide-generating system in leukocytes via controlling gp91-phox gene expression. J. Immunol., **186**, 3015-3022.

Kikuchi, H., Mimuro, H. and Kuribayashi, F. (2018): Resveratrol strongly enhances the retinoic acid-induced superoxide generating activity via up-regulation of gp91-phox gene expression in U937 cells. Biochem. Biophys. Res. Commun., **495**, 1195-1200.

Kikuchi, H., Mimuro, H., Madhyastha, H. and Kuribayashi, F. (2019): Chalcone skeleton promotes transcription of gp91-phox gene but inhibits expression of gp91-phox protein, and hydroxyl groups in hydroxychalcones participate in the stable expression of gp91-phox protein. Microbiol. Immunol., **63**, 438-443.

Kikuchi, H., Harata, K., Madhyastha, H. and Kuribayashi, F. (2021): Ellagic acid and its fermentative derivative urolithin A show reverse effects on the gp91-phox gene expression, resulting in opposite alterations in all-trans retinoic acid-induced superoxide generating activity of U937 cells. Biochem. Biophys. Rep., **25**, 100891.

Kim, N., Yoo, H.S., Ju, Y.J., Oh, M.S., Lee, K.T., Inn, K.S., Kim, N.J. and Lee, J.K. (2018): Synthetic 3′, 4′-dihydroxyflavone exerts anti-neuroinflammatory effects in BV2 microglia and a mouse model. Biomol. Ther. (Seoul), **26**, 210-217.

Leri, M., Scuto, M., Ontario, M.L., Calabrese, V., Calabrese, E.J., Bucciantini, M. and Stefani, M. (2020): Healthy effects of plant polyphenols: molecular mechanisms. Int. J. Mol. Sci., **21**, 1250.

Liang, W., Huang, X. and Chen, W. (2017): The effects of baicalin and baicalein on cerebral ischemia: a review. Aging Dis., **8**, 850-867.

Liu, H., Dong, Y., Gao, Y., Du, Z., Wang, Y., Cheng, P., Chen, A. and Huang, H. (2016): The fascinating effects of baicalein on cancer: a review. Int. J. Mol. Sci., **17**, 1681.

Miyata, Y., Shida, Y., Hakariya, T. and Sakai, H. (2019): Anti-cancer effects of green tea polyphenols against prostate cancer. Molecules, **24**, 193.

Moghadam, E.R., Ang, H.L., Asnaf, S.E., Zabolian, A., Saleki, H., Yavari, M., Esmaeili, H., Zarrabi, A., Ashrafizadeh, M. and Kumar, A.P. (2020): Broad-spectrum preclinical antitumor activity of chrysin: current trends and future perspectives. Biomolecules, **10**, 1374.

Nabavi, S.F., Braidy, N., Gortzi, O., Sobarzo-Sanchez, E., Daglia, M., Skalicka-Wozniak, K. and Nabavi, S.M. (2015): Luteolin as an anti-inflammatory and neuroprotective agent: a brief review. Brain Res. Bull., **119**, 1-11.

Panday, A., Sahoo, M.K., Osorio, D. and Batra, S. (2015): NADPH oxidases: an overview from structure to innate immunity-associated pathologies. Cell. Mol. Immunol., **12**, 5-23.

Saraei, R., Marofi, F., Naimi, A., Talebi, M., Ghaebi, M., Javan, N., Salimi, O. and Hassanzadeh, A. (2019): Leukemia therapy by flavonoids: future and involved mechanisms. J. Cell. Physiol., **234**, 8203-8220.

Schlupper, D., Giesa, S. and Gebhardt, R. (2006): Influence of biotransformation of luteolin, luteolin 7-O-glucoside, 3′, 4′-dihydroxyflavone and apigenin by cultured rat hepatocytes on antioxidative capacity and inhibition of EGF receptor tyrosine kinase activity. Planta Med., **72**, 596-603.

Theoharides, T.C., Stewart, J.M., Hatziagelaki, E. and Kolaitis, G. (2015): Brain "fog," inflammation and obesity: key aspects of neuropsychiatric disorders improved by luteolin. Front. Neurosci., **9**, 225.

Yan, X., Qi, M., Li, P., Zhan, Y. and Shao, H. (2017): Apigenin in cancer therapy: anti-cancer effects and mechanisms of action. Cell Biosci., **7**, 50.

Zhou, Y., Zheng, J., Li, Y., Xu, D.P., Li, S., Chen, Y.M. and Li, H.B. (2016): Natural polyphenols for prevention and treatment of cancer. Nutrients, **8**, 515.