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RESEARCH ARTICLE

Modulation of NF-κB and FOXOs by baicalein attenuates the radiation-induced inflammatory process in mouse kidney

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Abstract

The bioactive flavonoid baicalein has been shown to have radioprotective activity, although the molecular mechanism is poorly understood *in vivo*. C57BL/6 mice were irradiated with X-rays (15 Gy) with and without baicalein treatment (5 mg/kg/day). Irradiation groups showed an increase of NF-κB-mediated inflammatory factors with oxidative damage and showed inactivation of FOXO and its target genes, catalase and SOD. However, baicalein suppressed radiation-induced inflammatory response by negatively regulating NF-κB and up-regulating FOXO activation and catalase and SOD activities. Furthermore, baicalein inhibited radiation-induced phosphorylation of MAPKs and Akt, which are the upstream kinases of NF-κB and FOXOs. Based on these findings, it is concluded that baicalein has a radioprotective effect against NF-κB-mediated inflammatory response through MAPKs and the Akt pathway, which is accompanied by the protective effects on FOXO and its target genes, catalase and SOD. Thus, these findings provide new insights into the molecular mechanism underlying the radioprotective role of baicalein in mice.

Keywords: *Baicalein, ionizing radiation, inflammation, NF-κB, FOXOs.*

Introduction

Radiotherapy is one of the most effective cancer treatments used to control tumour progression because it induces anti-proliferative and cytotoxic effects in tumour tissue [1]; however, it often causes adverse reactions in normal tissue [2]. Among the major organs, the kidney is the most sensitive organ to irradiation that causes oxidative stress and nephropathy [3]. Therefore, many compounds have been sought for radioprotection against ionizing radiation (IR)-induced damage to normal kidney tissue [4].

Baicalein is one of the major flavonoids present in *Scutellaria baicalensis* and is known to function as an antioxidant, anti-inflammatory and anti-hepatotoxic agent in both *in vitro* and *in vivo* conditions [5,6]. Treatment with baicalein has been reported to attenuate endothelium intimal hyperplasia by inhibiting inflammatory signalling pathways involving extracellular signal-regulated kinase (ERK), Akt and nuclear factor-κB (NF-κB) activities in vascular smooth-muscle cells [7]. Baicalin, a precursor of baicalein, has been reported to attenuate NF-κB and P-selectin expression in the liver and kidneys of rats with severe acute pancreatitis [8]. A recent study showed that γ-irradiation with baicalein reduces lipid and protein oxidation in rat liver [9]. However, the radioprotective effects of baicalein on the kidney and the mechanisms underlying its antioxidant and anti-inflammatory activities have not been fully explored.

Damaging effects of IR are generally mediated through the production of reactive species (RS) and an increase in RS levels induces cellular damage, thereby leading to the generation of lipid peroxidation products as well as a decrease in antioxidant enzymes such as

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catalase and glutathione (GSH) [10]. Some RS can also assume the role of secondary messengers in the intracellular signalling pathways that activate the expression of many inflammatory genes. For instance, in the IR-induced molecular response characterized by increased cyclooxygenase-2 (COX-2) level, inducible nitric oxide synthase (iNOS) and vascular adhesion molecule-1 (VCAM-1) expressions induced the activation of the transcription factor NF-κB [11]. With regard to the radiation-induced inflammatory genes, COX-2, nitric oxide (NO) and cell adhesion molecules are major factors that contribute to radiation injury in normal tissues [12]. Cell adhesion molecules, such as VCAM-1, initiate leukocyte infiltration to the sites of irradiation, which might lead to the acceleration and/or induction of parenchymal atrophy, fibrosis and necrosis [13]. The radiation-induced production of NO is a characteristic feature of activated macrophages, which induce iNOS as a part of the inflammatory process and radiation pneumonitis [14]. Selective COX-2 inhibition in mouse skin after irradiation with 50 Gy showed less dermal inflammation and decreased infiltration of monocytes and neutrophils in treated animals [15].

The key role played by NF-κB activation in the process of inflammation has been reported to be closely associated with a redox-sensitive signal cascade that includes MAPKs (ERK, c-Jun N-terminal kinase [JNK] and p38) [11] and Akt [16]. However, activation of the Akt signalling pathway has been known to reduce forkhead box-O (FOXO) transcription activity and is involved in cytoprotective effects against oxidative stress [17].

The FOXO transcription factor plays a major role in regulating various aspects of cell metabolism, cell-cycle progression, apoptosis and oxidative stress resistance. The FOXO group has four mammalian gene members: FOXO1, FOXO3a, FOXO4 and FOXO6, and plays a significant role in cytoprotection against oxidative stress by up-regulating the transcription of the antioxidant enzymes such as catalase, Mn-superoxide dismutase (SOD), Cu/ZnSOD and glutathione peroxidase [18,19].

Phosphorylation of FOXO proteins by Akt is a key regulatory mechanism of FOXO activity that occurs in response to oxidative stress or growth factors, thereby resulting in their cytoplasmic sequestration due to which the proteins are unable to regulate gene expression [20]. In addition, recent evidence suggests that FOXO mediates its anti-inflammatory effects via negative regulation of NF-κB. For instance, FOXO4 has been shown to inhibit NF-κB transactivation by a direct interaction with NF-κB in trinitrobenzene sulphonic acid injury-induced colitis models [21]. In addition, T-cells from FOXO3a-deficient mice displayed markedly increased spontaneous NF-κB activity concomitant with a decrease in IκBβ [22]. These results indicate that FOXO acts as an endogenous suppressor of NF-κB.

Although the precise relationship between FOXO and NF-κB in IR-induced inflammatory response is not firmly established, the function of FOXO on NF-κB activation following IR may play an important role in regulating cellular homeostasis as shown in the our current study.

In the current study, we examined the molecular mechanisms and events underlying the protective effect induced by baicalein treatment against IR-induced inflammatory responses. Our evidence revealed that the antioxidative baicalein suppresses IR-induced increases in COX-2, iNOS and VCAM-1 levels and maintains catalase and SOD activities by modulating the activity of NF-κB and FOXOs.

Materials and methods

Animals

Male C57BL/6 mice (aged 8–10 weeks) were obtained from Samtako (Osan, Korea). The animal protocol used in this study has been reviewed by the Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC) on their ethical procedures and scientific care and it has been approved. Mice were housed six per cage and acclimatized for 1 week before starting the experiment.

Procedures for irradiation and baicalein injection

The mice were divided into the following three groups (six animals/group): control (no irradiation and no baicalein), irradiation-treated and irradiation with baicalein treatment. Mice in the baicalein treatment group were intraperitoneally injected (5 mg/kg body weight/day) for 3 days before X-irradiation. For whole body irradiation (WBI), the anaesthetized mice were exposed to 15 Gy X-radiation using a Clinac 21 EX (Varian medical system, Palo Alto, CA) X-ray generator operating at an exposure rate of 6 Gy/min. Mice were killed at 24 h after irradiation and the kidneys were quickly removed and rinsed in ice-cold buffer (100 mM Tris, pH 7.4, 1 mM EDTA, 0.2 mM PMSF, 1 μM pepstatin and 2 μM leupeptin). The tissue was immediately frozen in liquid nitrogen and stored at −80°C. To obtain serum samples, the mice were decapitated and blood was drawn and allowed to clot at room temperature for 30 min before being centrifuged at 2,700 × *g* at 4°C for 20 min. The supernatant was collected as serum and analysed for creatinine and urea levels.

Preparation of kidney tissue samples

Two hundred milligrams of frozen kidney tissue homogenized in 1 ml of hypotonic lysis buffer (buffer A: 10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF,

1 μM pepstatin and 2 μM leupeptin) by using a tissue homogenizer for 20 s. The homogenates were kept on ice for 20 min and 125 μL of 10% Nonidet P (NP)-40 solution was added and mixed for 15 s. The mixture was centrifuged at 12,000 × *g* for 3 min at 4°C to separate a fraction containing pellet nuclei and cytosol. The pelleted nuclei were washed with 225 μL of buffer B (200 μL of buffer A plus 25 μL of 10% NP-40) and re-centrifuged at 12,000 × *g* for 1 min. The final pellets were re-suspended in 200 μL of buffer C (50 mM HEPES, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM PMSF and 10% glycerol), mixed for 30 min and centrifuged at 12,000 × *g* for 15 min. Protein concentrations were measured by performing the bicinchoninic acid assay and then the samples were stored at –80°C.

Reagents

Baicalein was purchased from Sigma (St. Louis, MO, USA). Bioxytech LPO-586 assay kit was obtained from Oxis Health, Inc. (Foster, CA, USA). A horseradish peroxide-conjugated secondary antibody was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Polyvinylidene difluoride (PVDF) membranes were obtained from Millipore Corporation (Bedford, MA, USA). All other chemicals were of the highest purity available and were obtained from Sigma.

Kindey function

The levels of creatinine and urea were measured by using commercially available assay kits from BioVision by using standard methods (Mountain View, CA, USA) according to the manufacturer's instructions.

Assessment of lipid peroxidation

To evaluate lipid perioxidation, malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) concentrations were determined using a Bioxytech LPO-586 assay kit (Oxis Health, Inc.). The kit uses a chromatogenic reagent that reacts with the lipid peroxidation products MDA and 4-hydroxynonenales, thereby yielding a stable chromophore with maximum absorbance at 586 nm.

Measurement of GSH levels

Twenty-five percent meta-phosphoric acid was added to 100 μl cytosol fraction and then centrifuged at 12 000 × *g* for 10 min and the supernatants were used in the assay. For GSH measurement, EDTA-containing (1 mM) phosphate buffer (50 mM) was added to the supernatants, followed by addition of *o*-phthalaldehyde (0.1 mg/ml) and was incubated for 20 min at room temperature. The GSH levels were measured using a fluorescence plate reader, GENios (Tecan Instruments, Salzburg, Austria), with excitation and emission wavelengths set at 360 and 485 nm, respectively [23].

Enzymatic activities

Catalase activity. Catalase activity was assayed according to the method described by Aebi [24]. The final concentrations in the cuvettes were 50 mM potassium phosphate, pH 7, 30 mM H_2O_2 and cytosolic homogenates (0.05–0.1 mg). The decrease in absorbance at 240 nm after addition of the substrate was followed spectrophotometrically.

SOD activity. SOD activity was measured using the xanthine oxidase-cytochrome *c* method as described by McCord and Fridovich [25]. The final concentrations in the cuvettes were 50 mM potassium phosphate, pH 7.8, 0.1 mM EDTA, 10 mM cytochrome *c*, 50 mM xanthine, 50 or 2 mM cyanide, 1 U catalase and cytosolic homogenates (0.05–0.1 mg). The reaction was initiated by the addition of 1 U xanthine oxidase. The inhibition of xanthine oxidase was followed spectrophotometrically at 550 nm. One unit of SOD activity was defined as the amount of enzyme that gave 50% inhibition of the control rate of cytochrome *c* reduction.

Western blot

Homogenized samples were boiled for 5 min with a gel-loading buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 10% 2-mercaptoethanol and 0.2% bromophenol blue) in a ratio of 1:1. Total protein-equivalents for each sample were separated by SDS-polyacrylamide gel electrophoresis using 10% acrylamide gels and bands were transferred to a PVDF membrane. The membrane was allowed to block at room temperature for 1 h and was then incubated with a specific primary antibody at 25°C for 4 h, followed by a horseradish peroxidase-conjugated secondary antibody at 25°C for 1 h. Antibody labelling was detected using Westzol Plus (iNtRon Biotechnology, Seongnam, Korea) and chemiluminescence Fluorchem^{TM}SP (Alpha Innotech Corporation, San Leandro, CA, USA).

Statistical analysis

For the results of Western blot, one representative blot was shown in figures. For other assays, the results are expressed as mean ± SE from triplicate assays with five separate experiments. The statistical significance of the difference between the groups was determined by single-factor ANOVA followed by the Fisher's Protected LSD post-hoc test. Values of $p < 0.05$ were considered statistically significant.

Results

Effect of baicalein on renal function

Creatinine and urea concentrations were studied to assess the effect of baicalein treatment on kidney function. Blood urea levels were elevated at 24 h after irradiation, while this elevation in urea levels was abolished in the baicalein-administered IR group. In contrast, creatinine levels did not change significantly at 24 h after irradiation (Figure 1).

Baicalein-mediated protective effect against IR-induced oxidative damage

Formation of lipid peroxidation products, MDA and HAE, and alteration of GSH redox status were selected as measures to assess the extent of IR-related oxidative damage, as shown previously [26]. To elucidate the protective effects of baicalein on MDA and HAE formation and decreased GSH levels by IR exposure, MDA and HAE and GSH levels in kidney homogenates were measured. As shown in Figure 2A, IR exposure significantly enhanced MDA and HAE formation, whereas baicalein attenuated IR-induced MDA and HAE formation in kidney homogenates. Furthermore, as shown in Figure 2B, intracellular GSH levels were significantly down-regulated by IR and baicalein pre-treatment provided significant protection against the reduction in GSH levels.

Modulation of IR-induced NF-κB and FOXOs activation by baicalein

The transcriptional activity of NF-κB is stimulated upon serine residue phosphorylation of its p65 subunit by various kinases. The phosphorylation sites were identified at Ser276 in the Rel homology domain [27] and at Ser529/Ser536 in the C-terminal transactivation domains [28]. To determine the inhibitory effects of baicalein on NF-κB activation at 24 h after WBI, we tested protein levels of NF-κB p65, p50 and phosphorylated p65 using Western blot. As shown in Figure 3A, IR exposure resulted in the activation of p65 and p50 and phosphorylation of p65 at Ser276

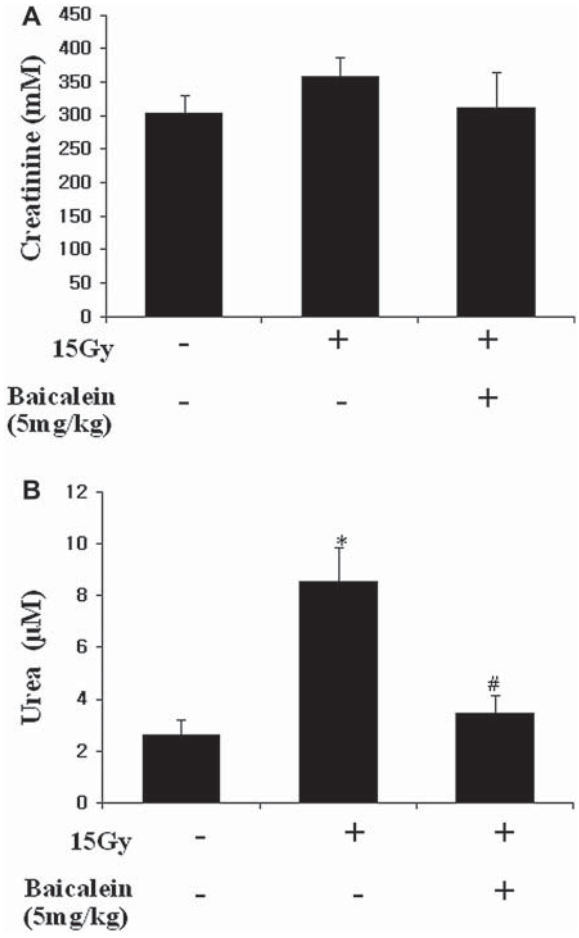

Figure 1. Effect of baicalein pre-treatment and/or irradiation on serum creatinine and urea. Levels of the kidney function indicators blood creatinine (A) and urea (B) were determined at 24 h after irradiation. As shown in the graph, values (mean ± S.E.) represented triplicate assays performed in six mice from each group ($n = 6$). Results of single-factor ANOVA followed by Fisher's protected LSD post-hoc test were used: $^{*}p < 0.05$ vs. non-exposed mice group; $^{\#}p < 0.05$ vs. IR-exposed mice group.

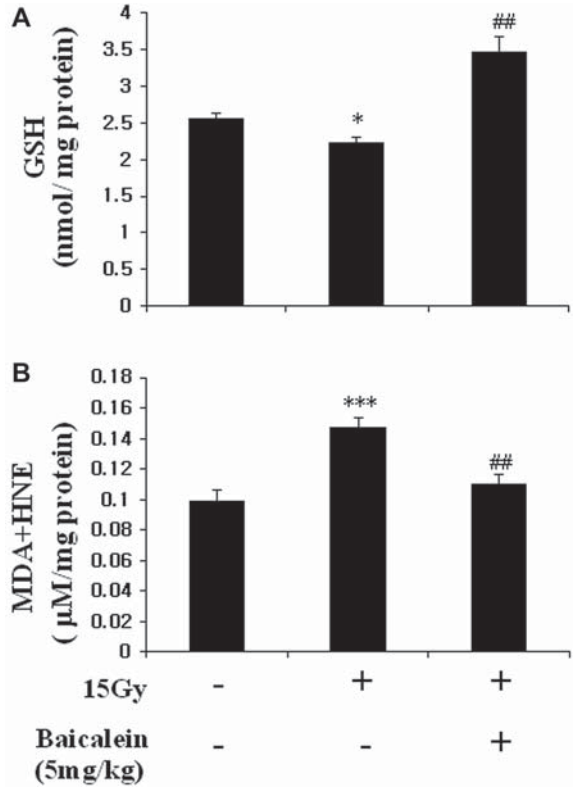

Figure 2. The influence of baicalein on MDA + HAE (B) and (A) GSH levels in IR-exposed mice kidney. MDA and HAE or GSH assays were performed as described in the methods section. Data obtained from mose kidneys at 24 h after IR are shown. As shown in the graph, values (mean ± S.E.) represented triplicate assays performed in six mice from each group ($n = 6$). Results of single-factor ANOVA followed by Fisher's protected LSD post-hoc test were used: $^{*}p < 0.05$, $^{***}p < 0.001$ vs. non-exposed mice group; $^{\#\#}p < 0.01$ vs. IR-exposed mice group, respectively.

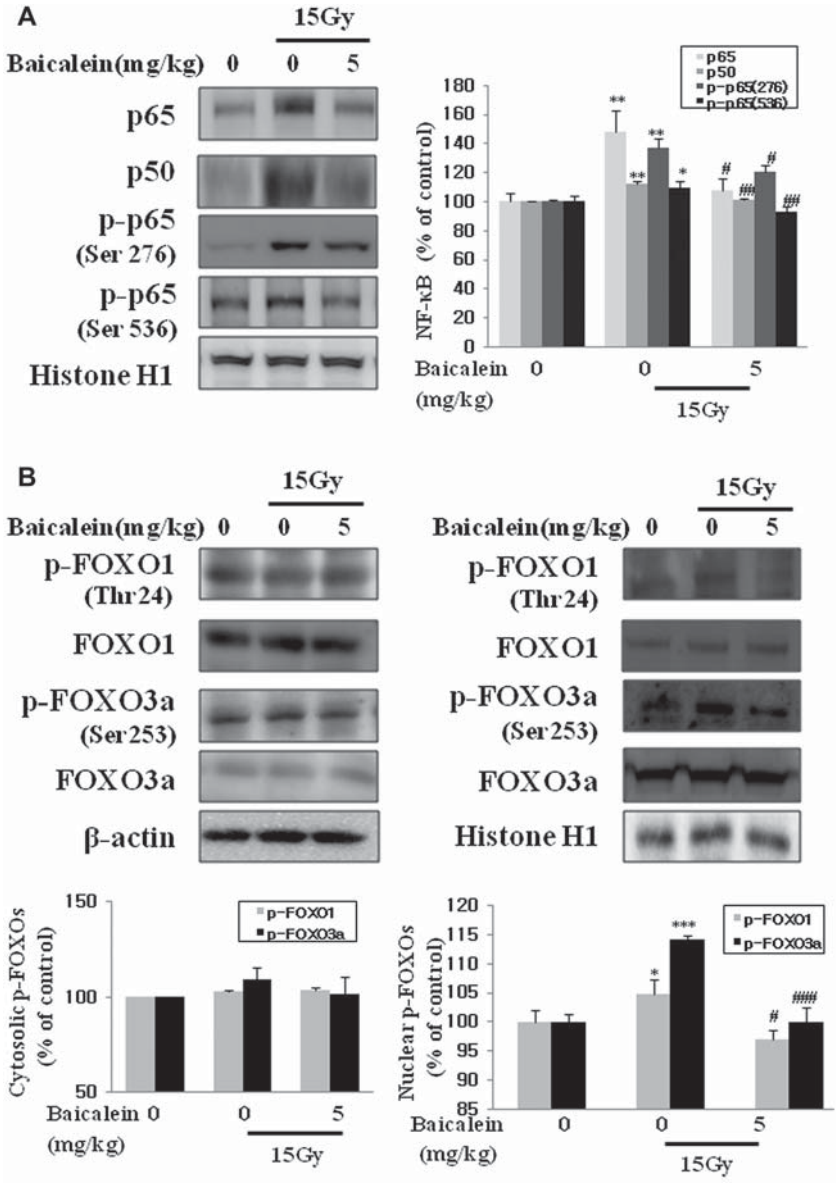

Figure 3. The effect of baicalein on IR-induced activation of NF-κB and FOXO. Mice were treated as described in the methods section. The activation of NF-κB p65, p50 and phospho-p65 (A) and FOXO1, FOXO3a, phospho-FOXO1 and phospho-FOXO3a (B) were determined using Western blot. One representative blot of each protein is shown from three experiments that yielded similar results ($n = 6$). Equivalent protein loading was confirmed by probing stripped blots for β-actin and histone H1. Results of single-factor ANOVA followed by Fisher's protected LSD post-hoc test were used: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. non-exposed mice group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. IR-exposed mice group.

and Ser536, while baicalein pre-treatment largely attenuated IR-stimulated activation of NF-κB.

Next, we elucidated changes in FOXO1 and FOXO3a in IR-induced mice homogenates and in the regulatory effects induced by baicalein. Phosphorylation of FOXOs results in the export of FOXO factors from the nucleus to the cytoplasm and in subsequent degradation by ubiquitination [20].

In the current study, we tested for the phosphorylated inactive state of FOXO1 and FOXO3a by performing Western blot in kidney cytosol and nuclear fractions. As shown in Figure 3B, the cytosol content of the phosphorylated FOXO1 (Thr24) and FOXO3a (Ser253) remained unchanged following IR, whereas the degree of nuclear compartmentalization increased. Baicalein suppressed IR-induced phosphorylation of nuclear FOXO1 and FOXO3a, thus enhancing the transcriptional activity of FOXOs. However, total FOXOs protein levels did not change. These data suggest that IR caused NF-κB activation and FOXO1 and FOXO3a inactivation due to increased oxidative damage and that baicalein inhibited these changes.

Suppressive action of baicalein on the expression of NF-κB and FOXO responsive proteins

Because we observed that baicalein regulates the activation of NF-κB and FOXOs in IR-induced

mice kidneys, we wanted to determine whether baicalein could inhibit the induction of NF-κB-responsive pro-inflammatory genes, such as COX-2, iNOS and VCAM-1, or increase the expression and enzymatic activities of FOXO-dependent antioxidant genes, catalase and Mn-SOD. As shown in Figure 4A, exposure to IR markedly enhanced the expression of those pro-inflammatory genes, but baicalein treatment inhibited them. In contrast, baicalein attenuates the IR-induced decrease in catalase and SOD protein levels (Figure 4B). Interestingly, however, Cu/ZnSOD expression was not altered by irradiation and/or baicalein treatment. Furthermore, our observations revealed that radiation decreased the enzymatic activities of catalase and SOD, whereas administration of baicalein with irradiation significantly increased the activities of the antioxidant enzymes catalase and SOD. Therefore, our data indicate that baicalein led to the inhibition of pro-inflammatory gene expression through NF-κB inactivation, while it increased the expression and enzymatic activities of antioxidant enzymes associated with FOXO activation.

Modulation of MAPKs and Akt signalling pathways by baicalein

To further investigate the inhibitory effect of baicalein on the upstream signalling pathways involved in NF-κB activation induced by IR exposure, we determined the phosphorylation status of ERK, p38 and JNK of the MAPK family and Akt by using Western blot. As

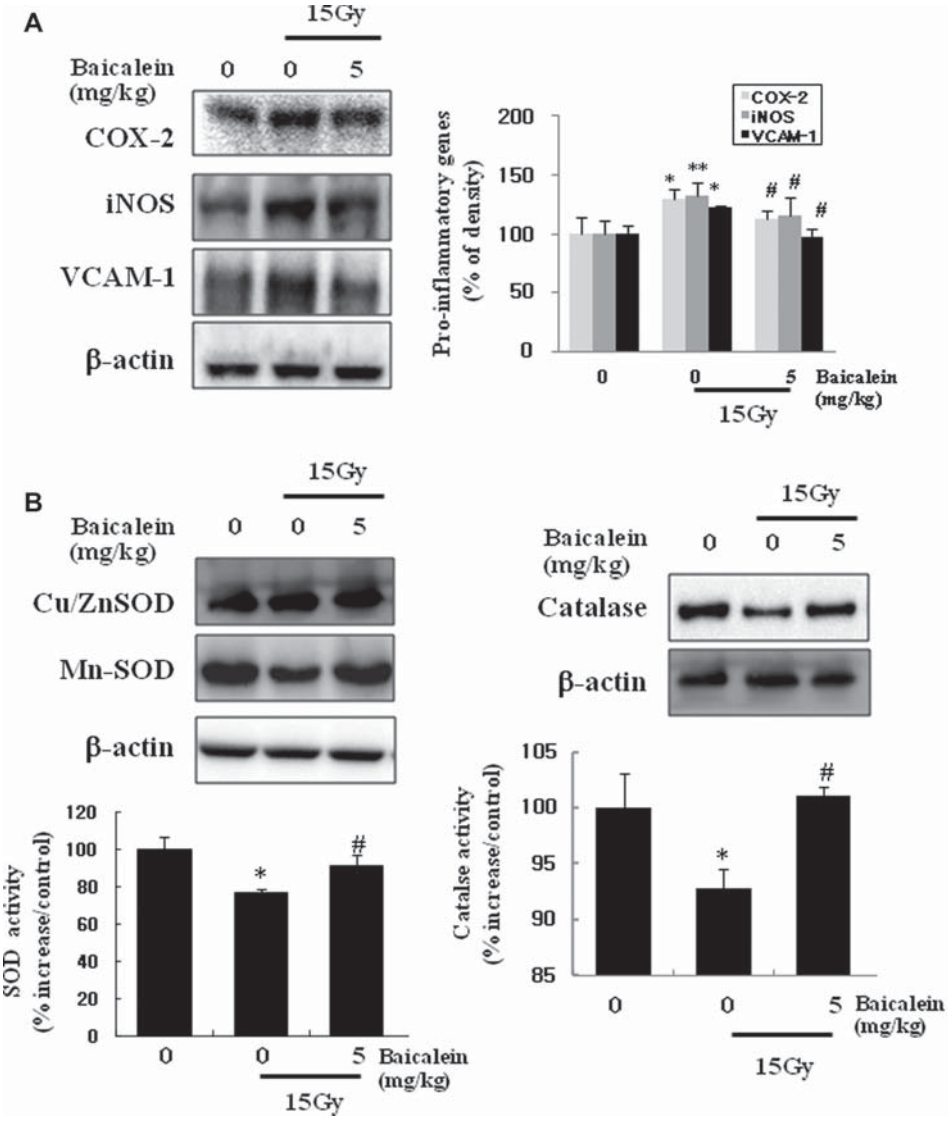

Figure 4. The effect of baicalein on IR-induced expression of pro-inflammatory and antioxidant genes. Kidney homogenates were prepared to determine the expression levels of the pro-inflammatory genes encoding COX-2, iNOS and VCAM-1 (A) and antioxidant genes encoding Cu/ZnSOD, Mn-SOD and catalase (B) using Western blot. A representative blot is shown from three independent experiments with identical observations ($n = 6$) and equivalent protein loading was confirmed by probing for β-actin. Activities of SOD and catalase were determined on kidney lysates as described in the methods section. Results of single-factor ANOVA followed by Fisher's protected LSD post-hoc test were used: $^{*}p < 0.05$, $^{**}p < 0.01$ vs. non-exposed mice group; $^{\#}p < 0.05$ vs. IR-exposed mice group.

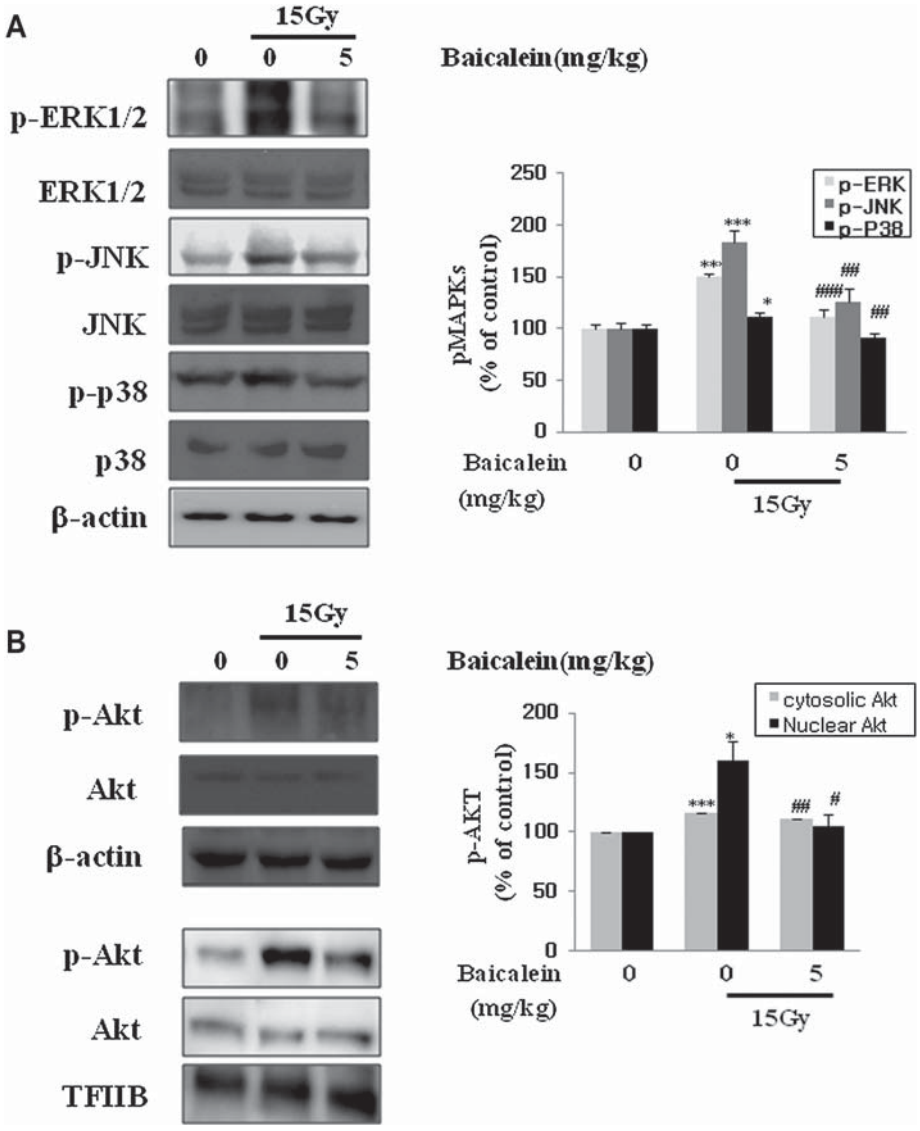

Figure 5. The inhibitory effects of baicalein on IR-exposed phosphorylation of MAPKs and Akt. Cytosolic and nuclear proteins were prepared to determine the phosphorylated levels of ERK 1/2, JNK and p38 of the MAPKs (A) and Akt (B) using Western blot. A representative blot is shown from three independent experiments with identical observations ($n = 6$) and equivalent protein loading was confirmed by probing stripped blots for total ERK1/2, JNK, p38, β-actin and transcription factor II B (TFIIB). Result of single-factor ANOVA followed by fisher's protected LSD post-hoc test were used: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. non-exposed mice group; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. IR-exposed mice group.

shown in Figure 5A, the phosphorylation of ERK, p38 and JNK increased following IR, whereas baicalein pre-treatment inhibited their phosphorylation. Furthermore, the phosphorylation of Akt was found to have increased both in the cytosolic and in the nuclear fraction following irradiation, as compared with that in the non-irradiated control group, whereas baicalein treatment significantly suppressed the phosphorylation of both cytosolic and nuclear Akt levels (Figure 5B). These results indicate that radiation-mediated phosphorylated Akt translocated to the nucleus where it phosphorylates FOXOs and was then subsequently translocated to the cytoplasm for degradation through the ubiquitin proteasome pathway. In addition, baicalein suppressed NF-κB activation by inhibiting MAPKs and Akt phosphorylation in the kidney of IR-induced mice. In particular, the inhibition of Akt by baicalein might suppress phosphorylation of both FOXO1 (Thr24) and FOXO3a (Ser253) in the nucleus.

Discussion

The purpose of the present study was, first, to establish whether baicalein pre-treatment can prevent the IR-induced pro-inflammatory process and, secondly, to delineate the molecular mechanisms and events underlying the modulation of redox sensitive pro-inflammatory transcription factor NF-κB and the cellular protective action mediated via FOXO activation. Our data clearly show that dietary baicalein supplementation

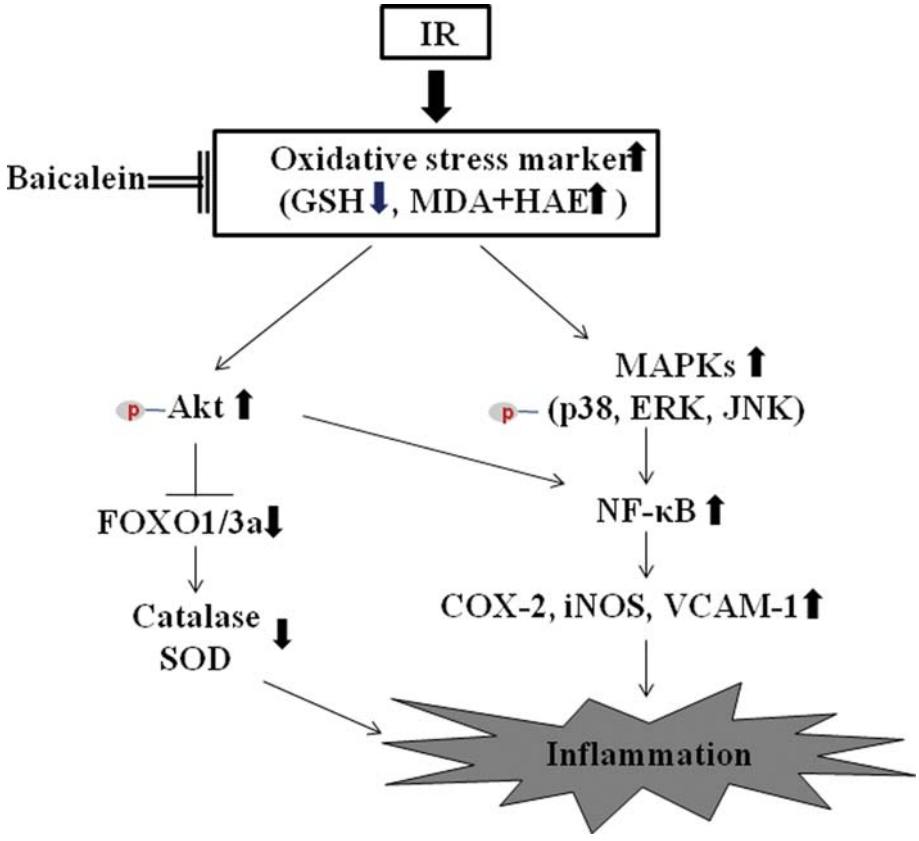

Figure 6. Outline of the modulation of IR-induced pro-inflammatory response by baicalein. In mouse kidneys, IR leads to increased MDA and HAE formation and depletion of intracellular GSH levels, which serve as an oxidative stress marker. Increases in IR-induced oxidative stress promote expression of pro-inflammatory mediators COX-2, iNOS and VCAM-1, all of which might contribute to augmented inflammation. Moreover, suppression of the activity of antioxidant enzymes such as catalase and SOD by IR exposure may amplify inflammatory responses. Furthermore, these mediators appear to be regulated by NF-κB activation through phosphorylation of MAPKs (p38, ERK and JNK) and Akt pathways as well as FOXOs inactivation through phosphorylated Akt following IR. Pre-treatment with baicalein, as an antioxidant phytochemical, regulates NF-κB and FOXOs signalling pathways through phosphorylation of MAPKs and Akt and prevents IR-induced oxidative stress. Thus, baicalein could inhibit the IR-induced pro-inflammatory response and the resultant renal inflammation.

effectively reduced IR-induced oxidative damage and inflammatory activation, as evidenced by the decrease of COX-2, iNOS and VACM-1, by negatively regulating NF-κB through the Akt and MAPKs signalling pathways. More significantly, our data further revealed that the down-regulation of Akt by baicalein led to FOXO activation and an increase in the activities of the FOXO-dependent genes encoding catalase and SOD, thus strengthening the radioprotective effects of baicalein against the inflammatory responses induced by IR (Figure 6).

NF-κB activation is a hallmark of cellular inflammatory responses to IR [11]. The transcriptional transactivation of NF-κB is modulated by various kinases through phosphorylation of NF-κB p65 [29]. In the present study, we obtained the evidence on marked increases in the phosphorylation of p65 at residues Ser276 and Ser536 through the up-regulation of upstream kinases MAPKs and Akt following IR, which was prevented by baicalein. Our findings are consistent with data showing that Ser276 of p65 is phosphorylated by the mitogen- and stress-activated protein kinase (MSK)-1 [30]. Thus, our data highlights that the transactivation function of NF-κB depends on the phosphorylation of Ser276 and Ser536 in IR-exposed mouse kidney and the inhibitory effect of baicalein on the IR-induced NF-κB modification.

Recently, Akt-FOXO signalling has been suggested as an important regulatory mechanism for FOXO expression in response to various stimuli [31]. In our study, the results indicate that irradiation enhances the p-Akt-mediated phosphorylation of FOXOs in the nucleus, after which the phosphorylated FOXOs are shuttled to the cytosol and serve as a target for the ubiquitin proteasome system, which prevents FOXO-DNA binding. Because poly-ubiquitination of FOXO results in its degradation, in our results, the levels of the phosphorylated form of FOXOs in the cytosol may not have changed because of irradiation. We also found that the total protein levels of FOXO1 and FOXO3a showed minimal differences in both the radiation-exposed and baicalein-treatment groups.

Although the radioprotective effect of FOXO has not been well-studied, there are a few studies showing that the beneficial action of FOXOs may be related to its anti-inflammatory activity. FOXO1 and FOXO3a have been identified as critical mediators of the cellular responses to oxidative stress and have been implicated in many ROS-regulated processes [32]. A recent study has reported FOXO as a target of the protective mechanism of flavonoids against the resistance to a lethal thermal stress [33]. Anti-angiogenic effects of resveratrol enhanced FOXO recruitment to the FOXO-binding element through deacetylation of FOXO1 [34]. In our current study, although the exact nature of the cross-talk between FOXO and NF-κB in IR-induced inflammatory responses has not been explored, we found that the inactivation of FOXO1 and FOXO3a by phosphorylation at Thr24 and Ser253, respectively, is associated with decreased catalase and SOD activity in activated NF-κB signalling. The antioxidant enzymes, catalase and SOD known to be expressed by the binding of FOXO, peroxisome proliferator-activated receptor (PPAR) and NF-E2-related factor 2 (Nrf-2) to their promoter regions [35,36]. Therefore, the FOXOs activation and the increased antioxidant SOD and catalase activity observed in our results seem closely associated, although the causal relation is hard to establish.

What makes the current study more interesting is the finding that baicalein not only significantly attenuated the radiation-induced inactive phosphorylated forms of FOXO1 and FOXO3a but also increased the activity and gene expression of the FOXO-target antioxidant enzymes, catalase and SOD, without changing the protein levels of Cu/ZnSOD. It is worth mentioning that Han et al. [37] showed that γ-radiation had no significant effect on Cu/ZnSOD expression in mice spleen and certain SODs are selectively induced by various stimuli such as cytokines [38], LPS [39] and anticancer agents [40].

Baicalein exhibits the highest free radical-scavenging activity among many flavonoid components [41] and many reports have shown its protective effect against oxidative stress-induced inflammatory response in various normal cells [41,42]. Baicalein, which has 12- and 15-lipoxygenase inhibitor function, shows cardiovascular protective action against oxidative stress-induced cell injury [42]. A previous study showed the protective effects of 5-lipoxygenase inhibitors against the tissue damage and NF-κB activation occurring after irradiation [43]. Considering these reports and our findings, we speculate that the radioprotective effect of baicalein may be partly related to its capacity to block lipoxygenase activity.

Interestingly, recent evidence has demonstrated baicalein's action as a pro-oxidant in inhibiting growth and proliferation through the generation of RS in cancer cells [44]. Similarly, resveratrol was also shown to induce apoptotic cell death in HL60 human leukaemia cell lines but not in normal peripheral blood lymphocytes [45]. In view of the above findings, we propose that plant polyphenol-mediated apoptosis is closely related to an increase in the concentrations of RS, which is possibly generated through the reduction of transition metals in cells [46].

Since irradiation increases the urea levels in serum, the protective action of dietary baicalein on the kidney was further evidenced by reduced levels of serum urea [47]. Although the process by which baicalein protects the kidney could be multi-factorial, the antioxidative action of baicalein, which suppresses the inflammatory process, could play a major role, as documented in the present study.

In summary, our results suggested that baicalein is a potent anti-radioprotective compound, with specific molecular properties to manipulate pro-inflammatory NF-κB and anti-inflammatory FOXO during IR-induced inflammatory response. In particular, baicalein, which up-regulated the enzymatic activity of catalase and SOD, might have played a central role in protection against the acceleration of the inflammatory response following IR. In conclusion, our study elucidates the underlying molecular mechanism and provides insights into the ability of bicalein to attenuate radiation-induced renal inflammation.

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Declaration of interest

This was supported for two years by Pusan National University Research Grant. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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