Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 2 pp. 203–211, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

NUTRITIONAL CONCENTRATION OF GENISTEIN PROTECTS HUMAN DERMAL FIBROBLASTS FROM OXIDATIVE STRESS-INDUCED COLLAGEN BIOSYNTHESIS INHIBITION THROUGH IGF-I RECEPTOR-MEDIATED SIGNALING

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Abstract: The effects of genistein, a soy isoflavone phytoestrogen and antioxidant, on collagen and DNA biosynthesis (measured by the 5-[^{3}H]proline and the [^{3}H]thymidine incorporation assays), prolidase activity (colorimetric method) and expression (determined by Western immunoblot) of the β1-integrin receptor, focal adhesion kinase pp125^{FAK} (FAK), Src, the insulin-like growth factor-I (IGF-I) receptor, Shc, growth-factor receptor-bound protein 2 (Grb2), son of sevenless protein (Sos) and phosphorylated mitogen activated protein (MAP) kinases, extracellular signal-regulated kinase 1 and 2 (ERK1/ERK2) were examined in normal human dermal fibroblasts (CRL-1474) exposed to oxidative stress. Subconfluent cells were subjected to repetitive stress with 30 μM *t*-butylhydroperoxide (*t*-BHP) in combination with 1-100 μM genistein for 1 h per day over the course of 5 days. Also, the cells were treated with *t*-BHP alone or with *t*-BHP in combination with 1-100 μM ascorbate. It was found that genistein at 1 μM counteracted the inhibition of collagen biosynthesis evoked by *t*-BHP in fibroblasts, more effectively than ascorbate at the same concentration. At 10 μM, genistein exerted significantly diminished protective effect on collagen biosynthesis in fibroblasts, while at 100 μM it induced inhibition of this process. The protective effect of genistein on collagen biosynthesis was not related to modulation of prolidase activity or the expression of the β1-integrin receptor, FAK, Src or Grb2. It was found that genistein, at 1 μM, diminished *t*-BHP-induced down-regulation of the IGF-I receptor, Shc, Sos and phosphorylated ERK1/ERK2 expression in fibroblasts. Simultaneously, genistein counteracted the antiproliferative activity of the oxidant. These results suggest that the mechanism of the protective effect of genistein on collagen biosynthesis in *t*-BHP-treated fibroblasts may be due to prevention of disturbances in the IGF-I receptor-mediated, ERK1/ERK2-associated signaling pathway evoked by the oxidant.

Keywords: collagen, fibroblast, genistein, IGF-I receptor signaling, oxidative stress

Collagen is not only a structural component of the connective tissue, but it also plays an important role as a ligand for the integrin family of cell surface receptors. It is known that the interaction between cells and extracellular matrix proteins, e.g. collagen, regulates cytoskeleton reorganization (1), intracellular ion transport, lipid metabolism, kinase activation, gene expression (2), cell cycle regulation (3) and cancer metastasis (4). Therefore, changes in the quantity, structure and distribution of collagen may affect cellular metabolism and function.

At least in fibroblasts collagen biosynthesis is under regulation of β1-integrin (5), IGF-I receptor signaling (6) and prolidase activity (7, 8). Prolidase [EC 3.4.13.9] is a cytosolic enzyme that catalyzes the hydrolysis of imidodipeptides (9), releasing proline, which is used for collagen resynthesis and cell growth (10, 11). Prolidase activity is known to be up-regulated by β1-integrin receptor (8). Stimulated β1-integrin receptor initiates a signaling cascade, which involves focal adhesion kinase pp125^{FAK} (FAK) (12), growth-factor receptor-bound protein 2 (Grb2), Src, Shc, son of sevenless protein (Sos), Ras and Raf proteins and leads to activation of the mitogen activated protein (MAP) kinases, extracellular signal-regulated kinase 1 and 2 (ERK1/ERK2) (13). Stimulation of the IGF-I receptor leads to activation of ERK1/ERK2 independently of FAK and Src (14).

Oxidative stress is known factor that affect collagen biosynthesis in tissues. It was documented a drastic inhibition of collagen biosynthesis in human dermal fibroblasts subjected to different oxidative stress inducers, including *t*-BHP (15), hydrogen peroxide and xanthine (16). In our previous paper, we showed that inhibition of collagen biosynthesis, induced by *t*-BHP in human dermal fibroblasts, may

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result from disturbances in the IGF-I receptor-mediated, ERK1/ERK2-associated signaling pathway (17). We hypothesized that the effects of the oxidant could be limited by the protective action of dietary antioxidants, such as genistein.

Genistein is natural isoflavonoid that besides its well-established antioxidant activity (18, 19), evokes a wide variety of biological effects through which it may affect collagen biosynthesis. These effects include modulation of estrogen and IGF-I receptor signaling (20, 21), inhibition of tyrosine kinases (22) and ribosomal S6 kinase (23) activities as well as activation of aminoacyl-tRNA synthetase (24).

The present study was undertaken to investigate the effects of genistein on collagen biosynthesis and the signaling pathways involved in its regulation in human dermal fibroblasts, under the conditions of oxidative stress evoked by *t*-BHP. We determined the effects of genistein as well as ascorbate, standard antioxidant on collagen and DNA biosynthesis; prolidase activity; and the expression of β1-integrin and IGF-I receptors as well as signaling molecules induced by their activation, i.e. FAK, Src, Shc, Grb2, Sos and phosphorylated ERK1/ERK2.

EXPERIMENTAL

Materials and methods

Polyclonal antibodies against FAK, human IGF-I receptor, goat immunoglobulin, mouse immunoglobulin, monoclonal antibody against phosphorylated ERK1/ERK2 and v-Src, L-ascorbate, bacterial collagenase (*Clostridium histolyticum*), 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent, genistein, L-glycyl-proline, L-proline and Nonidet P-40 were from Sigma Corp., USA, as were most other chemicals and buffers used. Human dermal fibroblasts (CRL-1474) were purchased from the American Type Culture Collection, Rockville, MD, USA. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were the products of Gibco, USA. Glutamine, penicillin and streptomycin were obtained from Quality Biologicals Inc., USA. *t*-Butylhydroperoxide (*t*-BHP) was the product of Fluka Chemie AG. Nitrocellulose membrane (0.2 mm), sodium dodecylsulfate, polyacrylamide, molecular weight standards and Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories, USA. 5-[^{3}H]Proline (28 Ci/mmol) was the product of Amersham, UK. Monoclonal (mouse) anti-Sos, anti-Grb2 and anti-Shc antibodies were obtained from Becton Dickinson Co., USA. Monoclonal (goat) anti-β1-integrin receptor antibody and polyclonal (goat) anti-β-actin antibody were from Santa Cruz Biotechnology Inc., USA. Polyclonal (rabbit) anti-human prolidase antibody was a gift from Dr. James Phang (National Cancer Institute at Frederick, MD, USA). Anti-rabbit immunoglobulin antibody was from Promega Corp., USA. [^{3}H]Thymidine (6.7 Ci/mmol) was purchased from ICN Biomedicals, USA and Scintillation Cocktail „Ultima Gold XR" from Packard, USA.

Tissue culture

Fibroblasts were maintained in DMEM supplemented with 10% FBS, 2 mM glutamine, 50 U/mL penicillin, and 50 mg/mL streptomycin at 37^{o}C in a 5% CO_2 incubator. Cells were counted in a hemocytometer and cultured at 1×10^5 cells per well in 3 mL of growth medium in 6 well plates. Cells reached about 80% of confluence at day 3 and such cells were used for the assays. Cells were used in the 8th to 14th passages.

Induction of oxidative stress in human dermal fibroblasts

Sub-confluent cells were subjected for 5 repeated exposures to oxidative stress by treatment with 30 μM *t*-BHP for 1 h per day over the course of 5 consecutive days, according to the method described by Dumont et al. (15). DMEM containing 10% FBS supplemented with 30 μM *t*-BHP alone or in combination with varying concentrations of genistein or ascorbate was added to each well and plates were incubated at 37^{o}C for 1 h. After that time, medium containing *t*-BHP or *t*-BHP with the studied antioxidants was removed and cells were rinsed twice with DMEM and maintained in DMEM containing 10% FBS. The next 1 h treatment of the fibroblasts (with *t*-BHP or *t*-BHP combined with antioxidants) was performed after 24 h, in order to let the cells recover from the previous exposure to *t*-BHP. Control cultures were incubated similarly but in the absence of *t*-BHP and antioxidants. Cells were harvested 24 h after the last scheduled exposure to oxidative stress.

DNA synthesis

DNA synthesis in human dermal fibroblasts was evaluated by measurement of [^{3}H]thymidine incorporation, as described previously (25).

Collagen synthesis

Cells were labeled for 24 h with the 5-[^{3}H]proline (5 μCi/mL, 28 Ci/mmol). Its incorporation into

proteins was measured as described previously (26). Incorporation of the tracer into collagen was determined by digesting proteins with purified *Clostridium histolyticum* collagenase, according to the method of Peterkofsky et al. (27). The results are shown as combined values for the cell plus medium fractions.

Determination of prolidase activity

The activity of prolidase was determined according to the method of Myara et al. (28). Protein concentration was measured by the method of Lowry et al. (29). Enzyme activity was reported as nanomoles of released proline during one minute per mg of supernatant protein.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Slab sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed according to the method of Laemmli (30).

Western immunoblot analysis

After sodium dodecylsulfate polyacrylamide gel electrophoresis, the gels were allowed to equilibrate for 5 min in 25 mM Tris, 0.2 M glycine in 20% (v/v) methanol. The protein was transfered to 0.2 mm pore-sized nitrocellulose at 100 mA for 1 h by using an LKB 2117 Multiphor II electrophoresis unit. The nitrocellulose was incubated: with polyclonal antibody against human prolidase at a dilution 1 : 3000; with monoclonal antibody against the IGF-I receptor at a dilution 1 : 500; with monoclonal antibodies against the β1-integrin receptor, FAK, Src, and Shc at a dilution 1 : 1000; with monoclonal antibody against Sos at a dilution 1 : 250; with polyclonal antibody against β-actin and monoclonal antibodies against Grb2 and phosphorylated ERK1/ERK2 at a dilution 1 : 5000 in 5% dried milk in Tris-buffered saline containing Tween 20 (20 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 0.05% Tween 20) for 1 h. In order to analyze prolidase and FAK, a second antibody, alkaline phosphatase conjugated, anti-rabbit immunoglobulin (whole molecule) was added at a dilution 1 : 5000; in order to analyze Src, Shc, Grb2, Sos and phosphorylated ERK1/ERK2, a second antibody, alkaline phosphatase conjugated, anti-mouse immunoglobulin (whole molecule) was added at a dilution 1 : 7500 and in order to analyze the β1-integrin receptor, the IGF-I receptor and β-actin, a second antibody, alkaline phosphatase conjugated, anti-goat immunoglobulin (whole molecule) was added at a dilution 1 : 5000 in Tris-buffered saline containing Tween 20 and incubated for 60 min under gentle shaking. Then, the nitrocellulose was washed with Tris-buffered saline containing Tween 20 (5 times for 5 min) and subjected to 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent. The intensity of the bands was quantified by densitometric analysis using an apparatus for gel documentation Syngen UVI-KS400 I (CO, SA) with digital densitometry and Scion Image Software.

Statistical analysis

The results presented on Figures 1, 2 and 5 were analyzed by analysis of variance (ANOVA) and multiple comparisons were done to check statistical significance. The data were expressed as the mean value for 3 independent assays ± S. D. The statistical significance between means was verified by Sheffe's comparison test accepting $p < 0.05$ as significant. In case of experiments shown on Figures 3 and 4, the results represent the mean value of 6 pooled extracts (Western-blot) from 3 independent experiments quantified by densitometric measurement. The densitometric analysis represent the mean value from 3 measurements.

RESULTS

Collagen biosynthesis was measured in fibroblasts incubated with 30 μM *t*-BHP alone or in combination with varying concentrations of genistein or ascorbate for 1 h per day, repeated over the course of 5 days. As can be seen in Figure 1 in fibroblasts treated with *t*-BHP collagen biosynthesis was decreased to about 50% of the control. However, an addition of 1 μM genistein counteracted the inhibitory effect of oxidative stress on collagen biosynthesis. This protective effect was also observed in fibroblasts treated with *t*-BHP and ascorbate. In the cells treated with *t*-BHP and 1 μM, 10 μM and 100 μM of this antioxidant, collagen biosynthesis was found to be about 80%, 125% and 110% of the control, respectively. Interestingly, in fibroblasts treated with *t*-BHP and 10 μM and 100 μM genistein, collagen biosynthesis was decreased to about 60% and 40% of the control, respectively. Similar extent of inhibition was found in the cells treated with 10 μM and 100 μM of genistein alone (data not shown). It supports previous report that genistein at high concentrations may inhibit collagen biosynthesis (31).

Genistein and ascorbate slightly affected prolidase activity in *t*-BHP-treated fibroblasts (Figure 2). In the cells incubated with *t*-BHP alone, prolidase

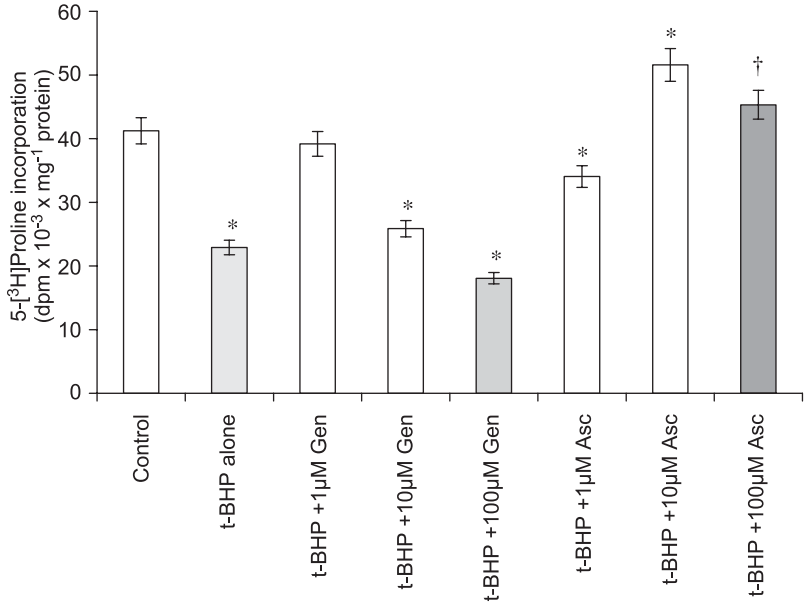

Figure 1. 5-[^{3}H]Proline incorporation into proteins susceptible to the action of bacterial collagenase in control human dermal fibroblasts and the cells treated with 30 µM t-BHP alone or with indicated concentrations of genistein (Gen) or ascorbate (Asc) for 1 h per day, repeated over the course of 5 days. Mean values from three independent experiments done in duplicates ± S.D. are presented. *$p < 0.001$, †$p < 0.05$.

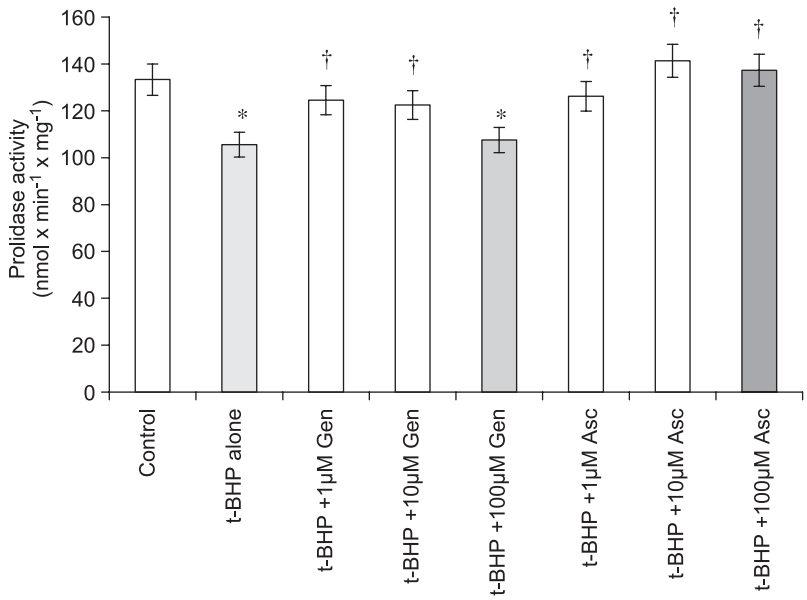

Figure 2. Prolidase activity in control human dermal fibroblasts and the cells treated with 30 ⌈M t-BHP alone or with indicated concentrations of genistein (Gen) or ascorbate (Asc) for 1 h per day, repeated over the course of 5 days. Mean values from three independent experiments done in duplicates ± S.D. are presented. *$p < 0.001$, †$p < 0.05$.

activity was decreased to about 80% of the control, whereas in cells treated with *t*-BHP and 1-10 µM genistein, the enzyme activity was decreased to about 90% of the control. Ascorbate at all studied concentrations revealed a similar protective effect on prolidase activity in *t*-BHP-treated fibroblasts. In case of the cells treated with *t*-BHP and 100 µM genistein, there was no effect on prolidase activity.

Prolidase activity is up-regulated through a signal mediated by β1-integrin receptor (8). However, we did not observe significant difference in β1-integrin receptor expression between control fibroblasts and

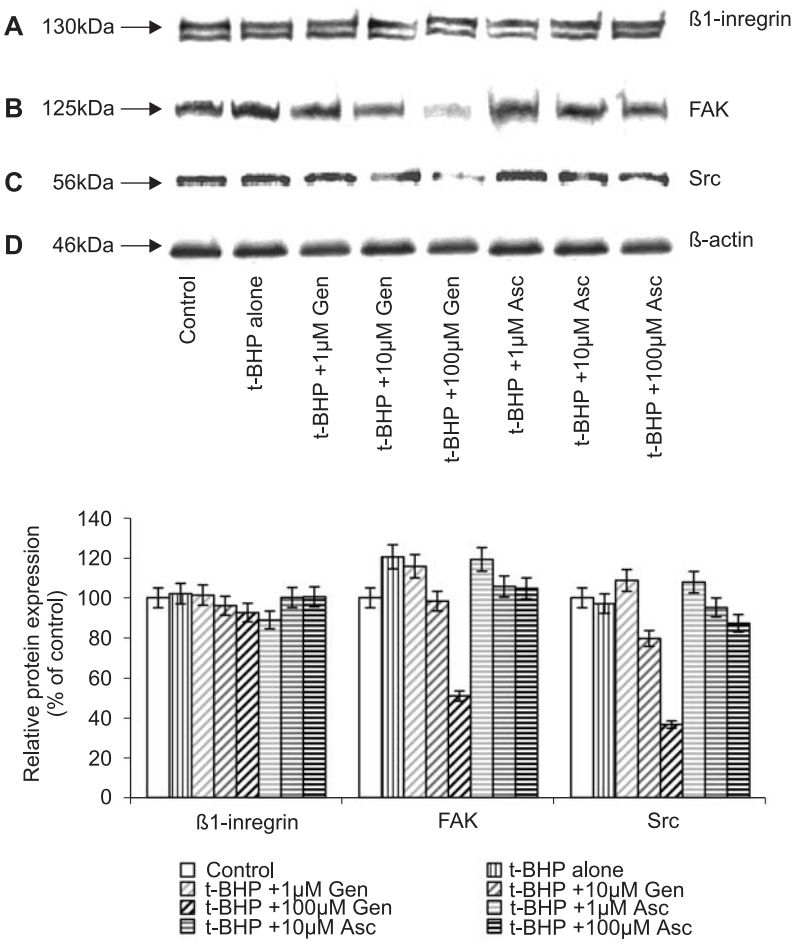

Figure 3. Upper panel: Western immunoblot analysis for the β1-integrin receptor (A), FAK (B), Src (C) and β-actin (D) in control human dermal fibroblasts and the cells treated with 30 µM *t*-BHP alone or with indicated concentrations of genistein (Gen) or ascorbate (Asc) for 1 h per day, repeated over the course of 5 days. This profile is representative of at least three different experiments. Samples used for electrophoresis consisted of 20 µg protein of pooled cell extracts (n=6). Detection of β-actin was carried out in order to provide the loading control. The arrows indicate the molecular mass of standards.
Lower panel: The intensity of the Western immunoblot bands was quantified by densitometric analysis. The relative amounts of proteins were normalized to β-actin levels.

the cells treated with either *t*-BHP alone, or *t*-BHP with varying concentrations of genistein or ascorbate (Figure 3A). Similarly, incubation of the cells with *t*-BHP alone, as well as in the presence of 1 µM genistein or 1-100 µM ascorbate, did not affect the expression of FAK or Src. However, an addition of genistein at 100 µM to the *t*-BHP-treated cells decreased the expression of FAK and Src to about 51% and 37% of the respective controls (Figures 3B and 3C).

Since IGF-I plays an important role in the regulation of collagen biosynthesis, the effect of genistein and ascorbate on the expression of the IGF-I receptor and signaling molecules induced by its activation in *t*-BHP-treated fibroblasts was studied. The activation of IGF-I receptor initiates a signaling pathway in which are involved Shc, Grb2, Sos, Ras and Raf proteins and phosphorylated ERK1/ERK2 (13). As can be seen in Figure 4A-E, the expression of IGF-I receptor, Shc, Sos and phosphorylated ERK1/ERK2, but not Grb2 in fibroblasts treated with *t*-BHP alone was significantly depressed to about 35%, 34%, 36% and 40% of the respective controls. Interestingly, in the cells treated with *t*-BHP and 1 µM, 10 µM or 100 µM genistein, the expression of IGF-I receptor was less affected, only to about 84%, 55% and 46% of the controls, respectively. At the same concentrations,

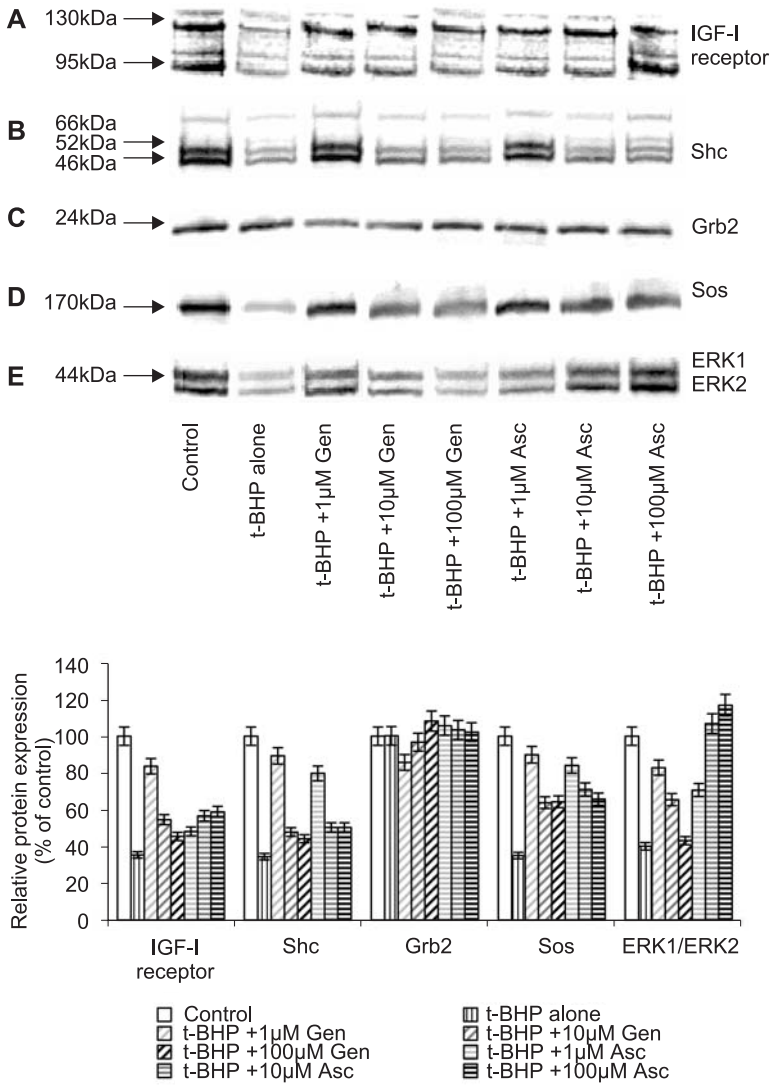

Figure 4. Upper panel: Western immunoblot analysis for the IGF-I receptor (A), Shc (B), Grb2 (C), Sos (D) and phosphorylated ERK1/ERK2 (E) in control human dermal fibroblasts and the cells treated with 30 µM *t*-BHP alone or with indicated concentrations of genistein (Gen) or ascorbate (Asc) for 1 h per day, repeated over the course of 5 days. This profile is representative of at least three different experiments. Samples used for electrophoresis consisted of 20 µg protein of pooled cell extracts (n = 6). The arrows indicate the molecular mass of standards.

Lower panel: The intensity of the Western immunoblot bands was quantified by densitometric analysis. The relative amounts of protein were normalized to β-actin levels.

ascorbate diminished the inhibitory effect of *t*-BHP on IGF-I receptor expression to about 48%, 57% and 59% of the controls, respectively (Figure 4A). Protective effects of both genistein and ascorbate were also observed in respect to Shc protein, however, at lower antioxidant concentrations. Expression of Shc in the cells treated with *t*-BHP and 1 µM genistein or ascorbate was found to be about 90% and 80% of the respective controls. In the cells treated with 10 – 100 µM of these compounds, the expression of Shc was decreased to about 45 – 50% of the controls (Figure 4B). Similar effect of both studied compounds was also found in respect to Sos protein. The inhibitory effect of *t*-BHP on Sos expression was diminished by 1 µM, 10 µM and 100 µM genistein or ascorbate to about 85%, 70% and 65% of the controls, respectively (Figure 4D). Interestingly, the above agents affected expression of MAP kinases in a quite differential manner. When genistein and ascorbate were added

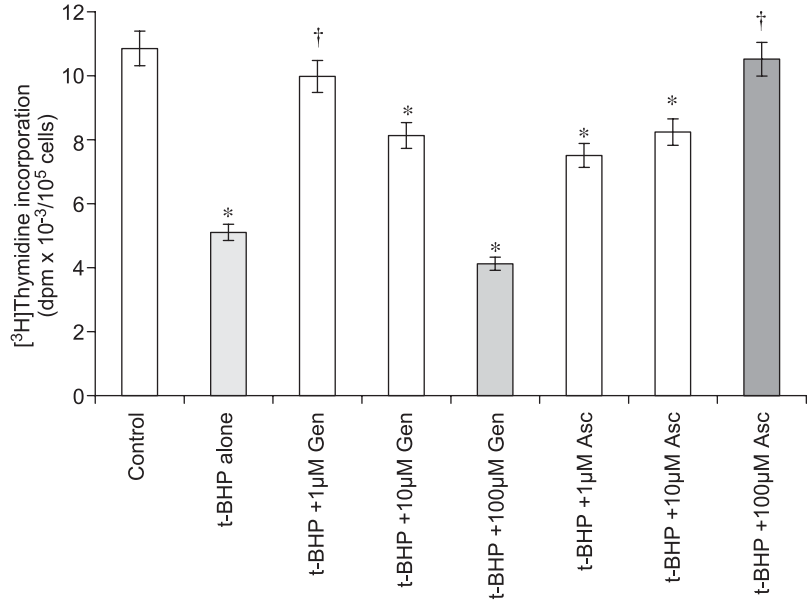

Figure 5. DNA biosynthesis (measured by the [^{3}H]thymidine incorporation assay) in control human dermal fibroblasts and the cells treated with 30 µM *t*-BHP alone or with indicated concentrations of genistein (Gen) or ascorbate (Asc) for 1 h per day, repeated over the course of 5 days. Mean values from three independent experiments done in duplicates ± S.D. are presented. Statistical significance is indicated in the figure: *$p < 0.001$, †$p < 0.05$.

to the *t*-BHP-treated cells at 1 µM, the expresssion of phosphorylated ERK1/ERK2 was recovered to about 83% and 71% of the controls, respectively. However, 10 µM or 100 µM concentrations of genistein suppressed phosphorylated ERK1/ERK2 to about 66% and 43% of the control. (Figure 4E). In case of ascorbate the same concentrations induced the expression of the MAP kinases to about 107% and 116% of the controls, respectively.

To determine the effects of genistein and ascorbate on DNA synthesis in fibroblast we performed the [^{3}H]thymidine incorporation assays. It is known that IGF-I receptor stimulation transmits mitogenic signals to the nucleus. As can be seen from Figure 5, DNA biosynthesis in fibroblasts treated with *t*-BHP alone was decreased to about 53% of the control. In the cells incubated with *t*-BHP and 1 µM or 10 µM genistein, this process was suppressed only to about 92% and 75% of the control, respectively. However, at concentration of 100 µM genistein, DNA biosynthesis was decreased to about 38% of the control. The protective effect of ascorbate on proliferation of the *t*-BHP-treated cells was found to be concentration dependent. In the cells treated with 1 µM, 10 µM or 100 µM of this antioxidant, DNA biosynthesis was found to be about 69%, 76% and 97% of the control, respectively These results were consistent with data of cell viability done by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay (data not shown).

DISCUSSION

Genistein is the isoflavonoid present in high levels in soybeans, and other plants from the *Papilionaceae* family. The concentration of genistein circulating in plasma of people regularly consuming soy products was found to be around 0.1 – 1.3 µM (32). Many epidemiological and animal model studies revealed potentially beneficial health effects of genistein. This phytoestrogen was shown to be an effective scavenger of free oxygen radicals (19, 33) and lipid peroxidation inhibitor (34). It is believed that the antioxidant property of genistein is especially important for the prevention of chronic diseases, such as cancer (35), cardiovascular disease (36), allergies and post-menopausal ailments (37).

Results of the present study suggest that under the conditions of oxidative stress induced in human dermal fibroblasts genistein (in contrast to ascorbate) exerts biphasic effects on collagen biosynthesis. Genistein, at 1 µM counteracted collagen biosynthesis inhibition evoked by *t*-BHP in fibroblasts, and was more efficient in this regard, than ascorbate at identical concentration. On the other hand, genistein, at 10 µM, exerted a significantly diminished protective effect on collagen biosynthesis, whereas at 100 µM, it potentiated the inhibitory action of *t*-BHP. Ascorbate (10-100 µM) appeared to be a strong inducer of collagen biosynthesis in *t*-BHP-treated cells. The effect of ascorbate possibly

resulted from stimulation of prolyl hydroxylase activity, the key regulatory enzyme in the process of collagen biosynthesis (38).

Another important enzyme in collagen biosynthesis is prolidase. However, genistein evoked little effect on prolidase activity in *t*-BHP-treated fibroblasts. Since drastic changes in 5-[^{3}H]proline incorporation evoked by different concentrations of genistein in *t*-BHP-treated fibroblasts were not accompanied by equivalent changes in prolidase activity, it suggests involvement of other mechanisms in the modulation of collagen biosynthesis by genistein.

Collagen biosynthesis and prolidase activity were shown to be up-regulated by the β1-integrin receptor-mediated signaling (5, 8). However, we observed no difference in expression of either this receptor or signaling molecules induced by its activation (FAK and Src), between fibroblasts treated with *t*-BHP alone or with 1 – 10 μM genistein and their respective controls. Only at 100 μM of genistein, there was a significant decrease in FAK and Src expression compared to their respective controls. This seems to be consistent with the results obtained by others (39, 40). These data suggest that the mechanism of the protective action of genistein on collagen biosynthesis in *t*-BHP-treated fibroblasts does not involve modulation of the β1-integrin receptor-mediated signaling.

Recently it was found that genistein enhances IGF-I receptor-mediated signaling in human breast cancer MCF-7 cells (21). The results of our study suggest that this isoflavonoid may exert similar effects in human dermal fibroblasts treated with *t*-BHP. Western immunoblot analysis showed a higher IGF-I receptor expression in cells treated with *t*-BHP and 1 μM genistein, compared to the cells treated with *t*-BHP alone. In addition, genistein at 1 μM was also found to exert a protective effect on the expression of phosphorylated ERK1/ERK2, as well as Shc and Sos proteins in *t*-BHP-treated cells. All these data support the idea that genistein may affect collagen biosynthesis through modulation of the IGF-I receptor-associated signaling pathway. It was recently demonstrated, that the activation of the IGF-I receptor protects cells from oxidative stress (41) and regulates their resistance to the action of oxidants (42). Furthermore, it was shown that genistein may stimulate IGF-I-mediated proteoglycans (43) and fibronectin synthesis (44) and may significantly enhance ERK1/ERK2 phosphorylation in fibroblast preincubated with fresh culture medium supplemented with this isoflavonoid (45). Therefore, it seems likely that the IGF-I receptor-mediated, ERK1/ERK2-associated signaling pathway could be the one, through which genistein exerts its protective effect on collagen biosynthesis in fibroblasts treated with *t*-BHP. This hypothesis is also supported by the results of the [^{3}H]-thymidine incorporation assays. Genistein, at 1 μM, was found to prevent DNA biosynthesis inhibition in *t*-BHP-treated cells more potently than identical concentration of ascorbate. It seems that genistein may evoke dual action, antioxidant and stimulatory on DNA synthesis. In fact, the stimulatory effect of low concentrations of genistein (up to 5 μM) on fibroblasts proliferation was shown in the absence of oxidants (46).

Nevertheless, genistein, at 100 μM, did not protect human dermal fibroblasts from collagen and DNA biosynthesis inhibition caused by *t*-BHP. Plasmid relaxation assays performed in our laboratory revealed that genistein, at 100 μM, exerted a significant inhibitory effect on topoisomerase II activity, whereas at 1 μM, genistein did not inhibit this enzyme activity (data not shown). These findings are consistent with the results obtained in other laboratories (47, 48). Suppression of topoisomerase II activity evoked by high concentrations of genistein may lead to disturbances in DNA synthesis and subsequently, transcription and translation. Furthermore, genistein is known to inhibit protein tyrosine kinases activity (22) and ribosomal S6 kinase activity (23), which could also be responsible for the lack of protective effects, at high concentrations of this compound, on collagen biosynthesis in *t*-BHP-treated cells.

Altogether, the results of the present study suggest that genistein, at nutritionally attainable concentrations (1 μM), protects human dermal fibroblasts from oxidative stress-induced collagen biosynthesis inhibition. The mechanism of the protective effect of genistein on collagen biosynthesis in *t*-BHP-treated fibroblasts may be due to prevention of disturbances in the IGF-I receptor-mediated, ERK1/ERK2-associated signaling pathway evoked by the oxidant.

Acknowledgments

We thank Dr. Christopher J. MacDonald (National Cancer Institute at Frederick, MD, USA) for his critical reading of the manuscript.

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Received: 2. 01. 2008