J. Biochem. 125, 910-915 (1999)

Serotonin Derivative, *N*-(*p*-Coumaroyl)serotonin, Isolated from Safflower (*Carthamus tinctorius* L.) Oil Cake Augments the Proliferation of Normal Human and Mouse Fibroblasts in Synergy with Basic Fibroblast Growth Factor (bFGF) or Epidermal Growth Factor (EGF)[1]

Takemasa Takii,* Masaru Hayashi,* Hiroyuki Hiroma,* Taku Chiba,* Sachiko Kawashima,* Hui Li Zhang,* Akito Nagatsu,* Jinsaku Sakakibara,† and Kikuo Onozaki*,[2]

**Faculty of Pharmaceutical Sciences, Nagoya City University, Mizuho-ku, Nagoya 467-0027; and* †*Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tempaku-ku, Nagoya 468-0077*

Received December 2, 1998; accepted February 5, 1999

N-(p-Coumaroyl)serotonin (CS) with antioxidative activity is present in safflower oil. We have reported that CS inhibits proinflammatory cytokine generation from human monocytes *in vitro*. As reactive oxygen species (ROS) affect cell proliferation, in this study the effect of CS on the proliferation of various cell types was examined. CS augments the proliferation of normal human and mouse fibroblast cells. The cells continue to proliferate in the presence of CS and form a transformed cell-like focus without transformation. CS, however, does not augment the proliferation of other cell types, either normal or tumor cells. CS augments the proliferation of fibroblasts in synergy with basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF), but not with acidic FGF(aFGF) or platelet-derived growth factor (PDGF). This study using synthesized derivatives of CS reveals that the growth-promoting activity is not due to antioxidative activity. These findings indicate that CS is a natural compound with unique growth-promoting activity for fibroblasts.

Key words: coumaroyl serotonin, EGF, FGF, fibroblast, growth factor.

Cell growth is regulated by a variety of factors, including growth factors, cytokines, hormones, and small molecular weight nutrients. Recent studies indicate that reactive oxygen species (ROS) mediate the signaling of several growth-regulatory factors including tumor necrosis factor α(TNFα), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) (*1-3*) and antioxidant/redox regulates gene expression (*4*). ROS generated by infiltrating macrophages, neutrophils, or within tissue cells also play an important role in tissue/cell injury and apoptosis (*5*). Therefore, ROS exert both positive and negative effects on cell proliferation.

Recently, we isolated potent antioxidative compounds from safflower (*Carthamus tinctorius* L) oil cake, a widely-used dietary oil (*6*). These compounds appeared to be N-(p-coumaroyl)serotonin (CS) (*7*) (Fig. 1) and its derivatives (*6, 8*). The antioxidative effect was at least partially due to radical scavenging activity (*8*). As ROS are generated from lipopolysaccharide (LPS)-stimulated macrophages and are involved in LPS signaling (*9, 10*), we examined whether CS inhibits proinflammatory cytokine generation from LPS-stimulated human monocytes *in vitro*. CS inhibits the production of TNFα, IL-1α, IL-1β, and IL-6 (*11*). In parallel, we examined the effect of CS on cell proliferation. In this study we demonstrate that CS is a novel growth promoting compound for normal human and mouse fibroblasts.

MATERIALS AND METHODS

Reagents—Eagle's MEM was purchased from Nissui Pharmaceutical (Tokyo). Fetal bovine serum (FBS) was from Sanko Junyaku (Tokyo). Female ICR mice (7 weeks of age) were purchased from SLC (Hamamatsu). Recombinant human aFGF and bFGF were from Pepro Tech EC (London, UK), PDGF was from Austral Biologicals (California, USA), and EGF was from Strathmann Biotech Gmbh (Hannover, Germany).

Isolation of CS—CS was isolated from safflower oil cake as previously reported (*6*).

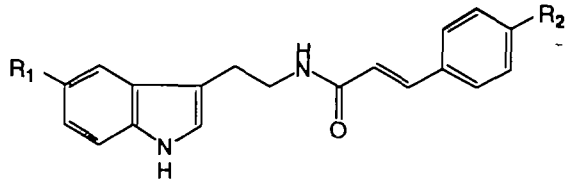
Cell Culture—Human lung fibroblast cell lines TIG-1,

[1] This work was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

[2] To whom correspondence should be addressed. Fax: +81-52-836-3419, E-mail: konozaki@phar.nagoya-cu.ac.jp

Abbreviations: Cin.S, N-(*trans*-cinnamoyl)serotonin; Cin.T, N-(*trans*-cinnamoyl)tryptamine; CS, N-(p-coumaroyl)serotonin; CT, N-(p-coumaroyl)tryptamine; EGF, epidermal growth factor; FBS, fetal bovine serum; FGF, fibroblast growth factor; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; HUVEC, human umbilical vein endothelial cells; IL, interleukin; LPS, lipopolysaccharide; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TNF, tumor necrosis factor.

MRC-5, MRC-9, and mouse fibroblast cell line Swiss 3T3 were obtained from the Japanese Cancer Research Resources Bank (Tokyo). The human melanoma cell line A375-C6 and mouse transformed fibroblast cell line L929 were maintained in this laboratory. The human breast carcinoma cell line MCF-7, and human promyelomonocytic cell line HL-60 were provided by Dr. Y. Endo and Dr. H. Henmi of Tohoku University (Sendai), respectively. The human hepatoma cell line HepG2 was obtained from the American Type Culture Collection. These cells were maintained in Eagle's MEM containing 100 units/ml of penicillin G, 100 μg/ml of streptomycin, and 10% heat-inactivated FBS. Mouse bone marrow cells and spleen cells were cultured in RPMI1640 (Sigma, SL, MO), 5×10^{-5} M 2-mercaptoethanol, 100 U/ml of penicillin G, 100 μg/ml of streptomycin, 15 mM HEPES, and 10% heat-inactivated FBS. Human umbilical vein endothelial cells (HUVEC) were cultured in MCDB104 liquid medium (Nissui Pharm., Tokyo) containing 5% FBS and a growth factor supplement.



CS $R_1 = R_2 = OH$
Cin. S $R_1 = OH$, $R_2 = H$
Cin. T $R_1 = R_2 = H$
CT $R_1 = H$, $R_2 = OH$

Fig. 1. **Structures of CS, Cin.S, Cin.T, and CT.**

Assay for Cell Proliferation—Adherent cells were detached from culture plates with 0.05% trypsin-0.02% EDTA to make a cell suspension. Cells suspended in culture medium containing 10% FBS and test samples were cultured in the wells of flat-bottomed 96-well microtiter plates (100 μl of 2 or 4×10^4 cell each; Falcon, Lincoln, NJ) at 37°C in air supplemented with 5% CO_2 for 3 days. CS and its derivatives were dissolved in dimethyl sulfoxide (DMSO) at 300 mM and diluted with culture medium. Proliferation of adherent cells, TIG-1, MRC-5, MRC-9, A375-C6, MCF-7, HepG2, Swiss 3T3, L929 cells, and HUVEC, was determined by a dye-staining method with crystal violet (*12*) or by counting the cell number using a hematocytometer. The proliferation of HL-60 cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (*13*), and that of mouse bone marrow cells and mouse spleen cells was determined by [^{3}H]thymidine incorporation assay. The proliferation of several cells, including L929 cells and HepG2 cells, was also determined by [^{3}H]thymidine incorporation assay.

[^{3}H]Thymidine Incorporation Assay—Mouse bone marrow cells and spleen cells were cultured for 4 days,

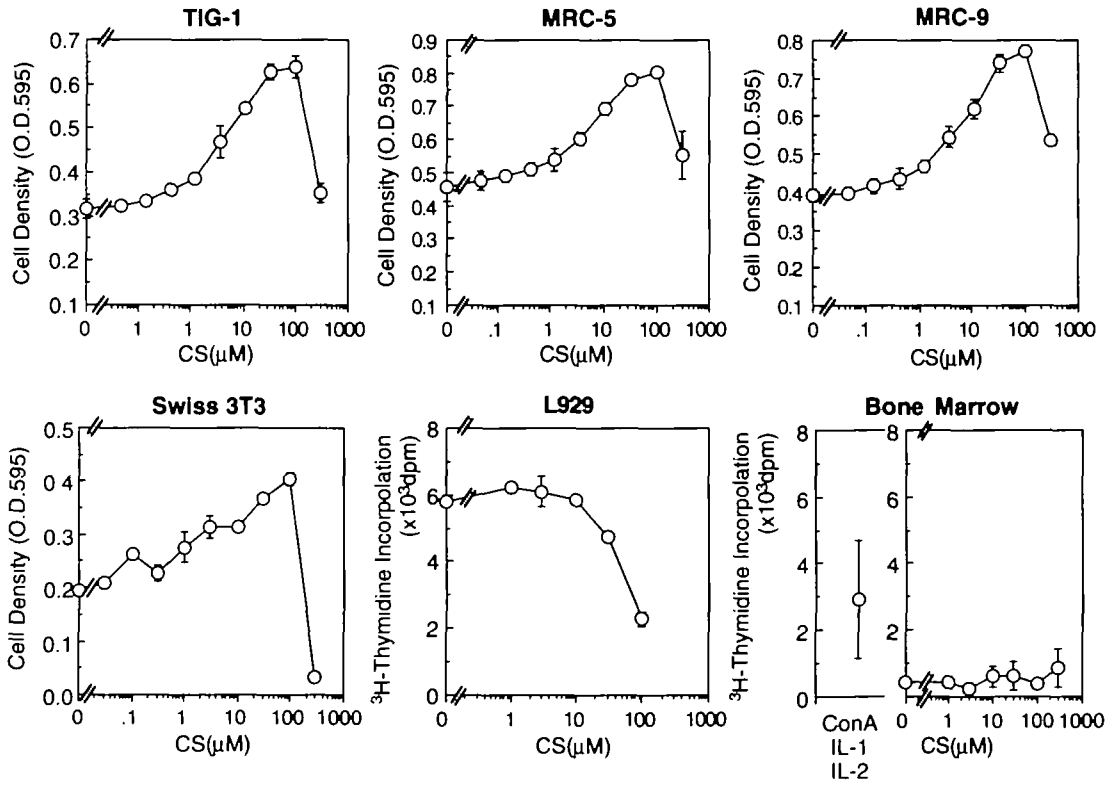


Fig. 2. **Growth-promoting activity of CS for normal human and mouse fibroblast cell lines.** TIG-1, MRC-5, MRC-9, and HepG2 cells (2×10^4/well) and Swiss 3T3 cells (4×10^4/well) were cultured with or without varying concentrations of CS in a 96-well plate for 3 days. Mouse bone marrow cells (5×10^4/well) were cultured with or without CS in a 96-well plate in the presence or absence of ConA (1.5 μg/ml), human IL-1α (2.5 U/ml), and human IL-2 (20 U/ml) for 4 days. Cell proliferation was determined by crystal violet staining or by [^{3}H]thymidine incorporation. Results are expressed as mean±SD based on triplicate cultures.

L929 cells and HepG2 cells were cultured for 3 days, and pulsed with [^{3}H]thymidine (1 μCi/well) for the final 18 h. Cells were collected with a cell harvester. The radioactivity incorporated into the cells was counted using a liquid scintillation counter (Aloka, Tokyo).

Synthesis of CS Derivatives—*N*-(*trans*-Cinnamoyl)serotonin (Cin.S) was synthesized by conjugating serotonin and cinnamic acid. *N*-(*trans*-Cinnamoyl)tryptamine (Cin. T) was synthesized by conjugating tryptamine and cinnamic acid. *N*-(*p*-coumaroyl)tryptamine (CT) was synthesized by conjugating tryptamine and *p*-coumaric acid (Fig. 1). A typical procedure was as follows. To a solution of dicyclohexylcarbodiimide (0.5 mmol) in tetrahydrofuran (THF, 5 ml), *p*-coumaric acid or cinnamic acid (each 0.5 mmol) was added, and the mixture was stirred at room temperature. After 1 h, serotonin or tryptamine (each 0.2 mmol) in THF (5 ml) was added, and the collection was stirred for 24 h at the same temperature. The whole mixture was filtered and the residue was washed with AcOEt. The combined filtrate and washings were concentrated to dryness to yield a crystalline residue that was purified on a column of silica gel eluted with $CHCl_3$–AcOEt–MeOH 8:10:1. The products were finally crystallized and identified by ^{1}H-NMR, ^{13}C-NMR spectral, and elemental spect analyses.

Antioxidation Assay—Antioxidative activity based on the inhibition of the generation of the peroxide of linoleic acid was performed by the ferric thiocyanate method as described previously (*8*).

RESULTS

Growth-Promoting Activity of Coumaroyl Serotonin for Normal Human and Mouse Fibroblast Cells—A variety of cell types were cultured with CS for 3 days, and the effect of CS on their proliferation was determined. The proliferation of three normal human fibroblast cell lines, TIG-1, MRC-5, and MRC-9, and a normal mouse fibroblast cell line, Swiss 3T3, were all augmented by CS in a dose-dependent manner over the concentration range of more than 5 μM up to 100 μM (Fig. 2). At higher concentrations, CS inhibited proliferation. However, CS did not augment the proliferation of other cells, L929 mouse transformed fibroblast cells or mouse bone marrow cells (Fig. 2), A375 human melanoma cells, HepG2 human hepatoma cells, MCF-7 human breast carcinoma cells, HL-60 human promyelomonocytic cells, mouse spleen cells, or HUVEC (data not shown). CS inhibited the proliferation of these cells at more than 100-300 μM because of its cytotoxicity, but cell proliferation was not affected by vehicle alone, serotonin, or coumaric acid (data not shown).

Continuous Proliferation of Fibroblast Cells in the Presence of CS—In order to determine whether CS augments the proliferation of normal human fibroblasts temporally or continuously, TIG-1 cells were cultured with and without CS by exchanging the medium every 3 days for up to 8 days (Fig. 3). TIG-1 cells have been widely used in studies of the responsiveness to cytokines, cell cycle analysis, and cellular senescence (*14, 15*). After 3 days culture, the proliferation of TIG-1 cells in medium alone was arrested and the cells showed contact inhibition. The cells did not grow even after exchanging the medium. However, in the presence of CS, the cells continued to proliferate. The cells kept growing in overlap and formed a

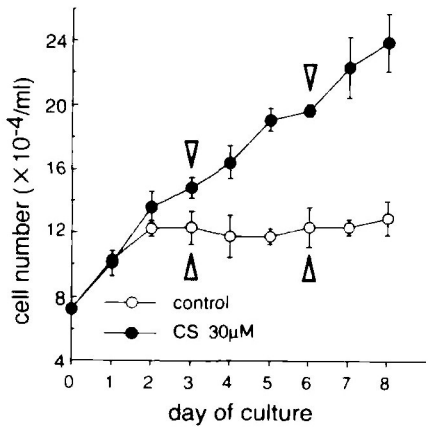


Fig. 3. **Continuous proliferative effect of CS on TIG-1 cells.** TIG-1 cells (7.2 · 10^4 cells well) in 1 ml medium were cultured in a 24-well plate with or without CS (30 μM). The medium was changed every 3 days. Proliferation was determined by counting the cells. Results are expressed as mean - SD based on triplicate cultures.

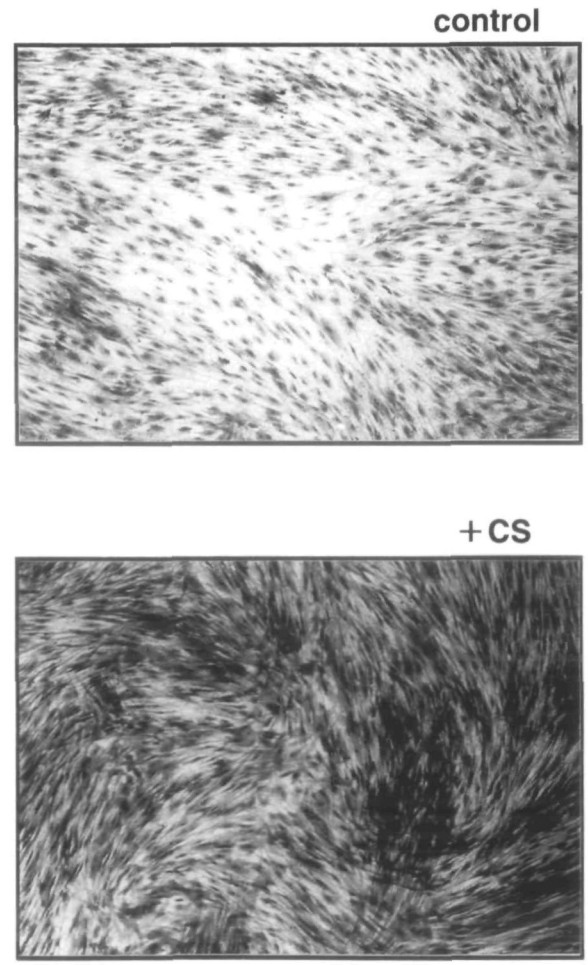


Fig. 4. **Cell morphology at day 3.** TIG-1 cells with or without CS (30 μM) were cultured for 3 days under the same culture conditions as in Fig. 2. The cells were stained with crystal violet and photographed (· 50).

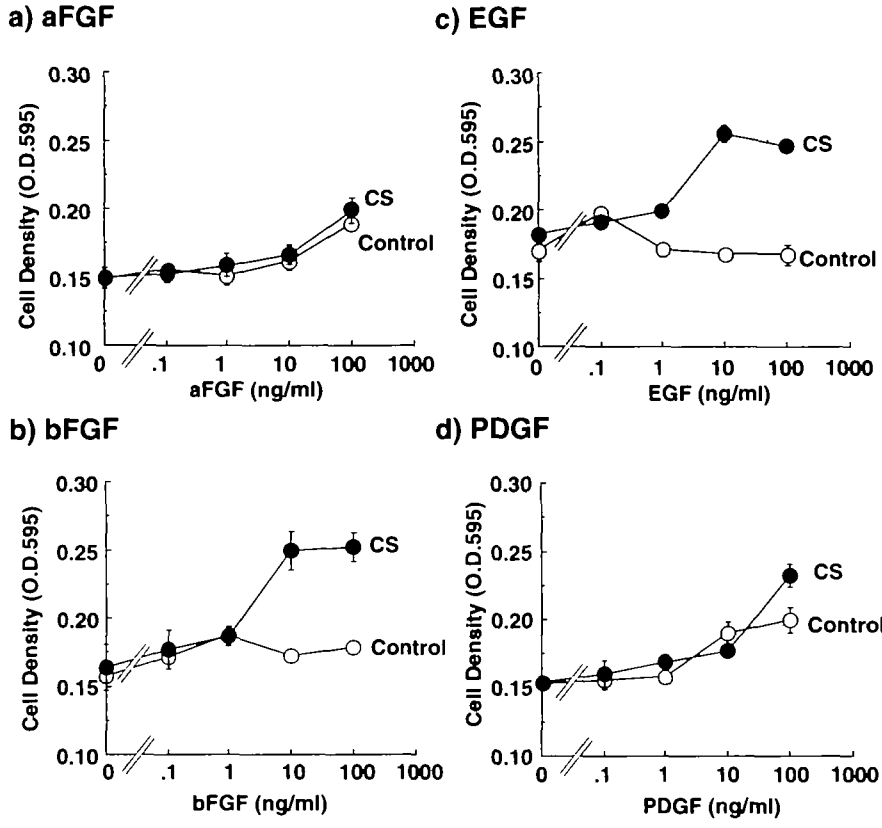


Fig. 5. **Synergistic effect of CS with growth factors.** TIG-1 cells in medium containing 0.5% FBS were cultured for 3 days with or without CS (30 μM) in the presence or absence of varying concentrations of aFGF (a), bFGF (b), EGF (c), or PDGF (d) in a 96-well plate. Results are expressed as mean ± SD based on triplicate cultures.

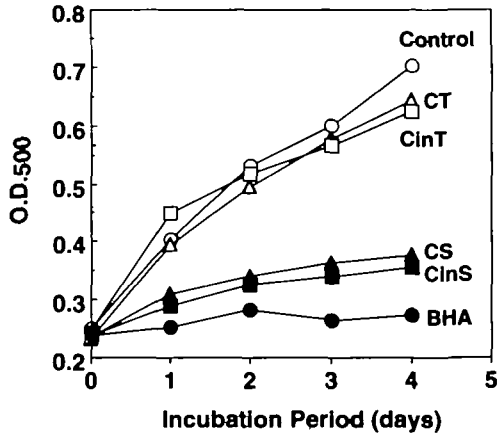


Fig. 6. **Antioxidative activity of CS and its derivatives.**

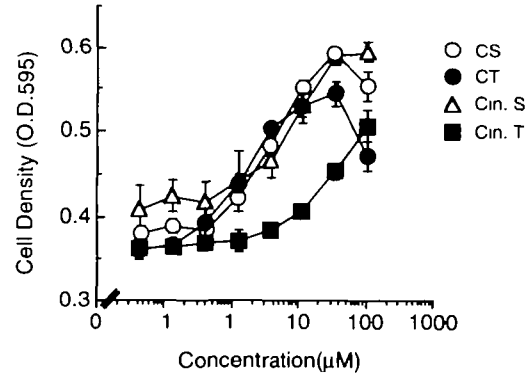


Fig. 7. **Growth-promoting activity of CS and its derivatives on TIG-1 cells.** Cells were cultured with or without varying concentrations of CS or its derivatives in a 96-well plate for 3 days. Cell proliferation was determined by crystal violet staining. Results are expressed as mean ± SD based on triplicate cultures.

transformed cell-like focus (Fig. 4). However, proliferation was arrested by removing CS, and cells cultured in fresh medium without CS after splitting exhibited contact inhibition (data not shown). These effects were also observed for MRC-5 and MRC-9 cells.

CS Augments Fibroblast Cell Proliferation in Synergy with bFGF or EGF, but Not with aFGF or PDGF—In order to determine whether CS shows a synergistic effect with growth factors, TIG-1 cells were cultured in medium containing 0.5% FBS in the presence or absence of aFGF, bFGF, EGF, or PDGF. As shown in Fig. 5, CS alone did not stimulate cell proliferation under these culture conditions. aFGF alone stimulated proliferation only at 100 ng/ml, and CS showed no synergistic effect with aFGF. bFGF or EGF alone had little or no stimulating effect on cell proliferation. However, CS showed a synergistic growth stimulating effect with bFGF or EGF. PDGF alone stimulated proliferation only at high doses. However, CS did not exhibit a synergistic effect with PDGF.

The Effect of CS Is Not Due to Antioxidative Activity—We synthesized three derivatives of CS, and determined their antioxidative activities by the ferric thiocyanate method using linoleic acid as a substrate for auto-oxidation in an ethanol-water system (Fig. 6). CT lacks the R_1 hydroxyl residue of serotonin, Cin.S lacks the R_2 hydroxyl

residue of coumaric acid, and Cin.T lacks both hydroxyl residues. The anti-oxidative activities of CS and Cin.S were comparable to that of BHA; however, CT and Cin.T lacked of activity. Therefore, the R_1, but not the R_2, hydroxyl residue appears to be important for antioxidative activity. When we examined the effect of CS derivatives on the proliferation of TIG-1 cells, Cin.S and CT augmented proliferation at levels comparable to that of CS. Cin. T also augmented proliferation, but the potency was about 10-fold less (Fig. 7). We also determined the effect of known antioxidative compounds, catalase, catechin, *N*-acetyl cysteine, 2-mercaptoethanol, and pyrrolidine dithiocarbamate (PDTC), on TIG-1 cell proliferation, but none showed an augmenting effect (data not shown).

DISCUSSION

In this study, CS appeared to augment the proliferation of normal human and mouse fibroblasts, but not other cell types, including normal and tumor cells. CS also exhibited synergistic effects with bFGF and EGF, but not with aFGF or PDGF. The refractoriness of tumor cells to CS is in good agreement with the concept that tumor cells produce autocrine growth factors such as FGF, tumor growth factor α (TGFα) and cytokines (*16, 17*), thus their growth occurs independent of exogenous growth factors. The normal cells we examined included mouse bone marrow cells, mouse spleen cells, and HUVEC. These normal cells require other types of growth factors, such as interleukin, colony stimulating factor (CSF), stem cell factor (SCF), or vascular endothelial growth factor (VEGF). Presumably, CS does not act in synergism with these growth factors. In support of this hypothesis, we observed no augmenting effect of CS in the IL-1-dependent murine T cell proliferation assay or IL-6-dependent hybridoma growth promoting assay (data not shown). So far as we examined, the CS growth-promoting effect is specific to normal fibroblasts. There has never been such a unique growth stimulating compound reported.

Normal fibroblasts exhibit contact inhibition. It is quite interesting that CS-treated fibroblast cells keep growing in overlap and form a transformed cell-like focus. However, this was not due to transformation because the CS effect is reversible and the fibroblasts did not grow in an anchorage-independent manner (data not shown). Transformation is reported to be associated with the induction of matrix metalloproteinase (MMP) (*18, 19*). In our study, however, none of the MMPs was induced by CS as determined by zymographic analysis (data not shown), supporting the conclusion that CS does not induce transformatiom. Contact inhibition is another reason why CS can not stimulate the proliferation of tumor cells. The growth of tumor cells can not be arrested in a confluent state, G1 phase, because cell cycle regulating molecules, including p53, p21, p15, p16, p18, and p19, are often mutated in tumor cells (*20–22*). The another interesting issue is whether CS is able to augment the proliferation of senescent cells. However, CS did not stimulate senescent fibroblast cells (data not shown).

The synergistic effect of CS with growth factors appears to be selective. In this study, aFGF exhibited a weak stimulating effect on TIG-1 cells relative to bFGF. This is not surprising because bFGF is reported to be 10 to 100-fold more potent than aFGF in its growth promoting effect *in vitro* (*23*). Therefore, aFGF at higher doses may also exhibit a synergistic effect with CS. In contrast to EGF and bFGF, CS did not exhibit a synergistic effect with PDGF. As PDGF has a moderate stimulating effect on cell proliferation, the lack of synergism between PDGF and CS is not due to a lack of a receptor for PDGF. The receptors for FGF, EGF, and PDGF are distinct molecules but with homologous tyrosine kinase domains in their cytoplasmic regions (*24*). A number of signaling pathways have been found downstream of each receptor. At present we do not know the mechanism of the synergistic effect. Whether CS augments the expression of receptors for FGF or EGF, or CS augments the signaling cascade of FGF or EGF remain to be elucidated. Recently, serotonin was reported to stimulate the tyrosine phosphorylation and proliferation of bovine pulmonary artery smooth muscle cells through the formation of O^{2-}, and its activity was found to be inhibited by several antioxidants (*25*). Although in our study serotonin was unable to stimulate the proliferation of target fibroblast cells, it is worthwhile to study whether CS stimulates the tyrosine phosphorylation of cells.

We considered that the growth-promoting effect of CS may result from its antioxidative activity because antioxidative activity and an inhibitory effect of CS on proinflammatory cytokine production from human monocytes were observed at similar doses between 10 to 100 μM (*8, 11*). Indeed, we have reported that catalase stimulates the proliferation of various cell types (*26, 27*). However, the effect of catalase was observed in many cell types, not only in normal cells but also in tumor cells, when the cell density was low. In this study, the CS augmenting effect was observed even when the cells were in a confluent state exhibiting contact inhibition. Then we synthesized several derivatives of CS, and examined their effects on fibroblast cell proliferation. The antioxidative and growth-promoting activities were not correlated. CT, which lacks antioxidative activity, augmented proliferation at a level comparable to CS. As Cin.S and CT possess growth promoting activities comparable to CS, and Cin.T shows about 1/10 the activity, the hydroxyl residue in either the R1 or R2 position appears to be important for the growth stimulating activity. In addition, known antioxidative compounds, including catalase, catechin, *N*-acetyl cysteine, 2-mercaptoethanol, and PDTC, all failed to promote the growth of TIG-1 cells. Therefore, the effect of CS is not due to its antioxidative activity.

CS is readily solubilized in oil. Therefore, although CS was isolated from safflower oil cake, substantial amounts of CS should be present in the oil, which is widely used in the diet. Previously, we reported that CS inhibits the production of proinflammatory cytokines, IL-1α, IL-1β, IL-6, and TNFα, as well as the activation of NF-κB in LPS-stimulated human monocytes (*11*). Thus, in conjunction with its antioxidative effect, CS may be beneficial to humans as an anti-inflammatory compound. As shown in this study, however, CS appears to have another interesting activity, a unique growth-promoting activity for normal fibroblasts. Thus, whether a daily intake of safflower oil can be benefit by stimulating the repair of tissue injury, or whether it is harmful because it augments fibroblast proliferation leading to fibrosis is another interesting issue to be studied. Finally, CS is a good tool for investigating the regulatory mechanism of fibroblast cell proliferation.

The authors thanks Dr. Hidetoshi Hayashi for helpful suggestions.

REFERENCES

1. Lo, Y. and Cruz, T.F. (1995) Involvement of reactive oxygen species in cytokine and growth factor of c-fos expression in chondrocytes. *J. Biol. Chem.* **270**, 11727-11730
2. Sundaresan, M., Yu, Z.X., Ferrans, V.J., Irani, K., and Finkel, T. (1995) Requirement for generation of H_2O_2 for platelet-derived growth factor signal transduction. *Science* **270**, 296-299
3. Bae, Y.S., Kang, S.W., Seo, M.S., Baines, I.C., Tekle, E., Chock, P.B., and Rhee, S.G. (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J. Biol. Chem.* **272**, 217-221
4. Sen, C.K. and Packer, L. (1996) Antioxidant and redox regulation of gene transcription. *FASEB J.* **10**, 709-720
5. Chapple, I.L. (1997) Reactive oxygen species and antioxidants in inflammatory diseases. *J. Clin. Periodontal.* **24**, 287-296
6. Zhang, H.L., Nagatsu, A., and Sakakibara, J. (1996) Novel antioxidants from safflower (*Carthamus tinctorius* L.) oil cake. *Chem. Pharm. Bull.* **44**, 874-876
7. Sakumura, S., Terayama, Y., Kawakatsu, S., Ichihara, A., and Saito, H. (1978) Conjugated serotonins related to cathartic activity in safflower seed. *Agric. Biol. Chem.* **42**, 1805-1806
8. Zhang, H.L., Nagatsu, A., Watanabe, T., Sakakibara, J., and Okuyama, H. (1997) Antioxidative compounds isolated from safflower (*Carthamus tinctorius* L.) oil cake. *Chem. Pham. Bull.* **248**, 1910-1914
9. Eugui, E.M., Delustro, B., Rouhafza, S., Iinicka, M., Lee, S.W., Wilhelm, R., and Allison, A.C. (1994) Some antioxidants inhibit, in a co-ordinate fashion, the production of tumor necrosis factor-α, IL-1β, and IL-6 human peripheral blood mononuclear cells. *Int. Immunol.* **6**, 409-422
10. Mendez, C., Garcia, I., and Maier, R.V. (1995) Antioxidants attenuate endotoxin-induced activation of alveolar macrophages. *Surgery* **118**, 412-420
11. Kawashima, S., Hayashi, M., Takii, T., Kimura, H., Zhang, H.L., Nagatsu, A., Sakakibara, J., Murata, K., Oomoto, Y., and Onozaki, K. (1998) Serotonin derivative, *N*-(*p*-coumaroyl)serotonin, inhibits the production of TNF-α, IL-1α, IL-1β, and IL-6 by endotoxin-stimulated human blood monocytes. *J. Interferon Cytokine Res.* **18**, 423-428
12. Ruff, M.R. and Gifford, G.E. (1980) Purification and physicochemical characterization of rabbit tumor necrosis factor. *J. Immunol.* **125**, 1671-1677
13. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. *J. Immunol. Methods* **65**, 55-63
14. Takii, T., Niki, N., Yang, D., Kimura, H., Ito, A., Hayashi, H., and Onozaki, K. (1995) Type I and type II interferons upregulate functional type I interleukin-1 receptor in a human fibroblast cell line TIG-1. *J. Interferon Cytokine Res.* **15**, 1065-1073
15. Fukami, J., Anno, K., Ueda, K., Takahashi, T., and Ide, T. (1995) Enhanced expression of cyclin D1 in senescent human fibroblasts. *Mech. Aging Dev.* **81**, 139-157
16. Goustin, A.S., Leof, E.B., Shipley, G.D., and Moses, H.L. (1986) Growth factors and cancer. *Cancer Res.* **46**, 1015-1039
17. Goldring, M.B. and Goldring, S.R. (1991) Cytokines and cell growth control. *Crit. Rev. Eukaryot. Gene. Exp.* **1**, 301-326
18. Himelstein, B.P., Lee, E.J., Sato, H., Seiki, M., and Muschel, R.J. (1997) Transformational activation of the matrix metalloproteinase-9 gene in an H-ras and v-myc transformed rat embryo cell line. *Oncogene* **24**, 1995-1998
19. Kadono, Y., Okada, Y., Namiki, M., Seiki, M., and Sato, H. (1998) Transformation of epithelial Madin-Darby canine kidney cells with p60(v-src) induces expression of membrane-type 1 matrix metalloproteinase and invasiveness. *Cancer Res.* **15**, 2240-2244
20. Kirsch, D.G. and Kastan, M.B. (1998) Tumor-suppressor p53: implications for tumor development and prognosis. *J. Clin. Oncol.* **16**, 3158-3168
21. Bissonnette, N. and Hunting, D.J. (1998) p21-induced cycle arrest in G1 protects cells from apoptosis induced by UV-irradiation or RNA polymerase II blockage. *Oncogene* **16**, 3461-3469
22. Drexler, H.G. (1998) Review of alterations of cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. *Leukemia* **12**, 845-859
23. Ibaraki, N., Lin, L.R., and Reddy, V.N. (1995) Effects of growth factors on proliferation and differentiation in human lens epithelial cells in early subculture. *Invest. Ophthalmol. Vis. Sci.* **36**, 2304-2312
24. Pouyssegur, J., Chambard J.C., L'Allemain, G., Magnaldo, I., and Seuwen, K. (1988) Transmembrane signaling pathways initiating cell growth in fibroblasts. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **320**, 427-436
25. Lee, S.L., Wang, W.W., and Fanburg, B.L. (1998) Superoxide as an intermediate signal for serotonin-induced mitogenesis. *Free Radic. Biol. Med.* **15**, 855-858
26. Takeuchi, A., Miyamoto, T., Yamaji, K., Masuho, Y., Hayashi, M., Hayashi, H., and Onozaki, K. (1995) A human erythrocyte-derived growth-promoting factor with a wide targer cell spectrum: Identification as catalase. *Cancer Res.* **55**, 1586-1589
27. Miyamoto, T., Hayashi, M., Takeuchi, A., Okamoto, T., Kawashima, S., Takii, T., Hayashi, H., and Onozaki, K. (1996) Identification of a novel growth-promoting factor with a wide target cell spectrum from various tumor cells as catalase. *J. Biochem.* **120**, 725-730