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Role of transforming growth factor-β pathway in the mechanism of wound healing by saponin from Ginseng Radix rubra

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1 The effects of saponin from Ginseng Radix rubra on extracellular matrix metabolism, the activation and synthesis of TGF-β1, and the modification of TGF-β receptor in fibroblasts were examined to elucidate the contribution of the TGF-β pathway to the mechanism of wound healing by saponin.

2 Fibronectin synthesis was analysed by the immunoprecipitation method. Activation and synthesis of TGF-β1 were measured by ELISA. The expressions of TGF-β receptors in fibroblasts were examined at protein and mRNA levels by the cross-linking method and Northern blot analysis, respectively.

3 The fibronectin synthesis increased 2.3- and 3.9-fold at fibroblasts treated with 1 and 10 μg ml^{-1} of saponin, respectively, compared with that in non-treated cells. Fibronectin synthesis stimulated with 10 μg ml^{-1} of saponin was inhibited with 69% by 5 μg ml^{-1} of an anti-TGF-β1 antibody. mRNA of TGF-β type I receptor increased 4.8- and 4.4-fold at fibroblasts treated with 1 and 10 μg ml^{-1} of saponin, respectively, and that of TGF-β type II receptor also increased 3.4- and 3.2-fold at fibroblasts treated with 1 and 10 μg ml^{-1} of saponin, respectively. The significant increases of TGF-β type I and II receptors and of fibronectin synthesis were observed at the same concentrations of saponin. TGF-β1 content increased 1.74- and 1.87-fold at conditioned medium of fibroblasts treated with 100 and 250 μg ml^{-1} of saponin, respectively, higher concentrations than those which accelerated fibronectin synthesis. Furthermore, the active TGF-β1 content was below 10% of total TGF-β1 at each concentration of saponin.

4 These results indicate that saponin stimulates fibronectin synthesis through the changes of TGF-β receptor expressions in fibroblasts.

Keywords: Ginseng; saponin; wound healing; fibroblasts; fibronectin; extracellular matrix; transforming growth factor-β (TGF-β; TGF-β receptor

Introduction

Ginseng Radix rubra has been clinically used in Oriental countries for treatment of various diseases, such as, atherosclerosis, cerebrovascular diseases, liver dysfunction, postmenopausal disorders and hypertension (Yamamoto, 1988). Saponin is the major active component of Ginseng Radix rubra and contains at least twenty identified ginsenosides (Yamamoto, 1988). In clinical trials, orally-administrated Ginseng Radix rubra stimulated the repair of intractable skin ulcers in patients with diabetes mellitus and Werner's syndrome (unpublished data). In an *in vivo* animal model, we have reported that local administration of saponin markedly improved wound healing in diabetic or aging rats (Morisaki *et al*., 1995).

The most important step of the initial would healing process is the formation of granulation tissue. The histological features of granulation tissue are characteristic in the proliferation of new small blood vessels (angiogenesis) and of fibroblasts. It is reported that saponin stimulated angiogenesis through the increase of protease activities and the migration of human endothelial cells (Morisaki *et al*., 1995). Fibroblasts in the granulation tissue synthesize the extracellular matrix, including glycoprotein, proteoglycan and collagens (Benet, 1985), but it is not clear what the effects of saponin are on the relation between fibroblasts and extracellular matrix synthesis during the process of wound healing. It is well known that extracellular matrix metabolism is controled mainly by transforming growth factor-β (TGF-β) *in vitro* and *in vivo* (Liau & Chan, 1989; Mustoe *et al*., 1987). Actually, Mustoe *et al*. (1987) reported that the breaking strength of a skin incisional wound in rat was increased by the administration of TGF-β to the wound, indicating that TGF-β accelerated the wound healing process *in vivo*. Therefore, it needs to be determined whether TGF-β contributes to the process of wound healing by saponin through extracellular matrix metabolism.

TGF-β is usually synthesized and secreted in a biological latent form (Pircher *et al*., 1984) and its function is transduced by its receptors, i.e., TGF-β type I and type II receptors (Lopez-Casillas *et al*., 1994; Wieser *et al*., 1993). In this study, we examined the effects of saponin on the extracellular matrix metabolism, the activation and synthesis of TGF-β1, and the modification of TGF-β receptor expressions in fibroblasts in order to clarify the contribution of the TGF-β pathway to the mechanism of wound healing by saponin.

Methods

Cell culture

Fibroblasts from human skins were cultured in Dulbecco's modified Eagles's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were subcultured at a split of 1:2 and used at the 4th–6th passages for the following experiments.

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Analysis of fibronectin synthesis in fibroblasts

To detect fibronectin synthesis, fibronectin in medium and cell lysates from ^{35}S-methionine and cysteine labeled fibroblasts was examined as described by Ignotz & Massague (1986). Briefly, confluent fibroblasts were incubated with serum-free DMEM with 0–500 μg ml^{-} of saponin for 20 h and then labeled with ^{35}S-methionine and cysteine in methionine- and cysteine-free DMEM with 0–500 μg ml^{-1} of saponin at 37°C for 3 h. When using an anti-TGF-β1 antibody, cells were incubated with serum-free DMEM with 10 μg ml^{-1} of saponin and various concentrations of antibody (0, 1, 2, 5 μg ml^{-1}) for 20 h and labeled. After 3 h labeling, the medium and lysates of cells were collected. 50 μl of gelatin-Sepharose suspension was added to the medium and lysates, followed by incubation at 4°C with constant agitation overnight. Gelatin-Sepharose beads were recovered by centrifugation and washed three times with 0.5% Triton X-100, 0.15 M NaCl, 25 mM Tris-HCl, pH 7.4. Fibronectin was eluted from the beads by boiling in SDS-sample buffer containing 4% SDS, 0.2 M Tris-HCl, pH 8.8, 0.5 M sucrose, 0.1% bromphenol blue and 2% β-mercaptoethanol, and was analysed by SDS-gel electrophoresis using 5% polyacrylamide gel, followed by fluorography.

Assay of TGF-β1 in conditioned medium (CM) of fibroblasts

Confluent fibroblasts in 6-well plates (Iwaki Glass, Funabashi, Japan) were incubated with 2 ml of serum-free DMEM for 24 h, and then were changed to fresh DMEM containing 0–500 μg ml^{-1} of saponin. After 24 h of incubation, the medium was centrifuged at 500 *g* and the supernatant was collected (CM). TGF-β1 was assayed in CM directly or CM treated at 80°C for 5 min with a human TGF-β1 immunoassay kit using anti-human TGF-β1 antibody and TGF-β type II receptor (R&D Systems, Minneapolis, MN, U.S.A.). TGF-β1 assayed in CM directly contains only active TGF-β1 but that in CM treated at 80°C shows total TGF-β1 containing active and latent TGF-β1 (Brown *et al*., 1990).

Detection of TGF-β receptor by cross-linking

Cross-linking experiments were performed as previously described (Ichijo *et al*., 1990). In brief, confluent fibroblasts in 6-well plates were incubated with serum-free DMEM with 0–500 μg ml^{-1} of saponin for 24 h, and then cells were washed with binding buffer (PBS containing 0.9 mM $CaCl_2$, 0.49 mM $MgCl_2$ and 1 mg ml^{-1}BSA) and incubated on ice in the same buffer with 100 pM ^{125}I-TGF-β1 in the presence or absence of excess unlabeled TGF-β1 for 3 h. Cells were washed, and cross-linking was done in the binding buffer without BSA together with 0.25 mM Bis for 30 min on ice. Supernatants from solubilized cells containing the same amounts of protein were subjected to analysis by SDS gel electrophoresis using 6% polyacrylamide gel, followed by autoradiography.

Detection of mRNAs for TGF-β receptor by Northern blot analysis

Northern blot analysis was carried out as described by Claesson-Welsh *et al*., (1988). mRNAs were purified from confluent fibroblasts incubated with serum-free DMEM containing 0–500 μg ml^{-1} of saponin for 24 h as described by Auffray & Rougeon (1980). Then, 2.5 μg of mRNAs from various conditioned fibroblasts were electrophoresed on 1% agarose gel in the presence of formaldehyde and blotted to Hybond N^{+} membrane (Amersham International plc, Buckinghamshire, U.K.). Northern hybridization was performed in 50% formamide, 5 × SSC (1 × SSC contains 15 mM sodium citrate and 150 mM NaCl, pH 7.4), 5 × Denhardt's solution, 0.1% SDS, 50 mM sodium phosphate buffer (pH 6.5), and 0.1 mg ml^{-1} of salmon sperm DNA at 42°C overnight with a human cDNA of TGF-β type I or II receptor labeled with the Megaprime DNA labeling system (Amersham) as a probe. The membrane was washed three times for 20 min for each time in 0.1 × SSC and 0.1% SDS at 42°C, dried, and visualized by autoradiography.

Statistical methods

The significance of differences was evaluated by ANOVA.

Materials

Sources of materials were as follows: FBS (Lot No. 37K0325), GIBCO Laboratories (Grand Island, NY, U.S.A.); anti-TGF-β1 antibody, R&D Systems (Minneapolis MN U.S.A.); ^{125}I-TGF-β1, [^{35}S]-methionine, [^{35}S]-cysteine and α-[^{32}P]-dCTP, New England Nuclear (Boston, MA, U.S.A.); Gelatin-Sepharose, Pharmacia LKB (Uppsala, Sweden); Bis (sulfosuccinimidyl) suberate, Pierce (Rockford, IIinois, U.S.A.). cDNAs of TGF-β type I and II receptors were kindly provided by Dr. Kohei Miyazono (Department of Biochemistry, The Cancer Institute, Tokyo, Japan).

Saponin was separated and purified as described by Kitagawa *et al*. (1989) and supplied by The Conference on Drug Ginseng. In brief, the extract from the air-dried root of Ginseng Radix rubra treated with 80% aqueous methanol was subjected to ethyl acetate/water separation. The extract of the water phase was adsorbed to a SEP-PAK C_{18} column, (Waters, Milford, MA, U.S.A.). After washing with 20% methanol, saponin was eluted with 100% methanol. This saponin contained more than 20 ginsenosides with similar structures (Kitagawa *et al*., 1983; Yamamoto, 1988).

Results

Effects of saponin on fibronectin synthesis of fibroblasts

Fibronectin is one of the important components in extracellular matrix and is known to play an essential role in the wound healing process (Colvin, 1989; Ruoslahti, 1988). We examined fibronectin metabolism in saponin-treated fibroblasts. Fibronectin synthesis was measured in medium and cell lysates of metabolically labeled fibroblasts. Bands around 220 kd corresponded to fibronectin, which was in agreement with the result of an earlier experiment using an anti-fibronectin antibody (Ignotz & Massague, 1986). The intensities of the fibronectin bands in the medium and lysates of fibroblasts increased dose-dependently with the addition of 1–10 μg ml^{-1} of saponin (Figures 1 and 2), but decreased with 100–500 μg ml^{-1} of saponin. The intensities of the bands in the medium increased 2.3- and 3.9-fold at fibroblasts treated with 1 and 10 μg ml^{-1} of saponin, respectively, compared with that without saponin treatment (Figure 1). Those in the lysates increased 2.0- and 2.2-fold at fibroblasts treated with 1 and 10 μg ml^{-1} of saponin, respectively (Figure 2). These increases were statistically significant.

Effects of anti-TGF-β1 antibody on fibronectin synthesis of fibroblasts

To clarify the contribution of TGF-β to increased fibronectin synthesis by saponin, we examined the effects of an anti-TGF-β1 antibody on fibronectin synthesis of fibroblasts. Fibronectin synthesis in conditioned medium of fibroblasts treated with 10 μg ml^{-1} of saponin was 3.9-fold that without saponin. Fibronectin synthesis stimulated with 10 μg ml^{-1} of saponin was inhibited dose-dependently by 1–5 μg ml^{-1} of the anti-TGF-β1 antibody (Figure 3). The inhibition of fibronectin synthesis by anti-TGF-β1 antibody was not observed in cells without saponin treatment. The maximal inhibition rate of fibroncetin synthesis was 69% at fibroblasts treated with 5 μg ml^{-1} of anti-TGF-β1 antibody, suggesting that the increased fibronectin synthesis by saponin is mainly due to the activated TGF-β pathway.

Effects of saponin on TGF-β1 content in CM of fibroblasts

The basal amount of total TGF-β1 in CM was 176±41.7 pg ml^{-1} (mean±s.d., n=10) in fibroblasts. The total TGF-β1 content in CM from cells treated with 1, 10, 100, 250 and 500 μg ml^{-1} of saponin increased 0.95±0.09-fold (mean±s.d., n=10), 1.18±0.42-fold (mean±s.d., n=10), 1.74±0.44-fold (mean±s.d., n=10), 1.87±0.06-fold (mean±s.d., n=10) and 1.63±0.64-fold (mean±s.d., n=10), respectively, compared with that from non-treated cells (Table 1). The increases were statistically significant at 100 and 250 μg ml^{-1} of saponin. However, active TGF-β1 content was below 10% of total TGF-β1 and did not increase by treatment with saponin (data not shown).

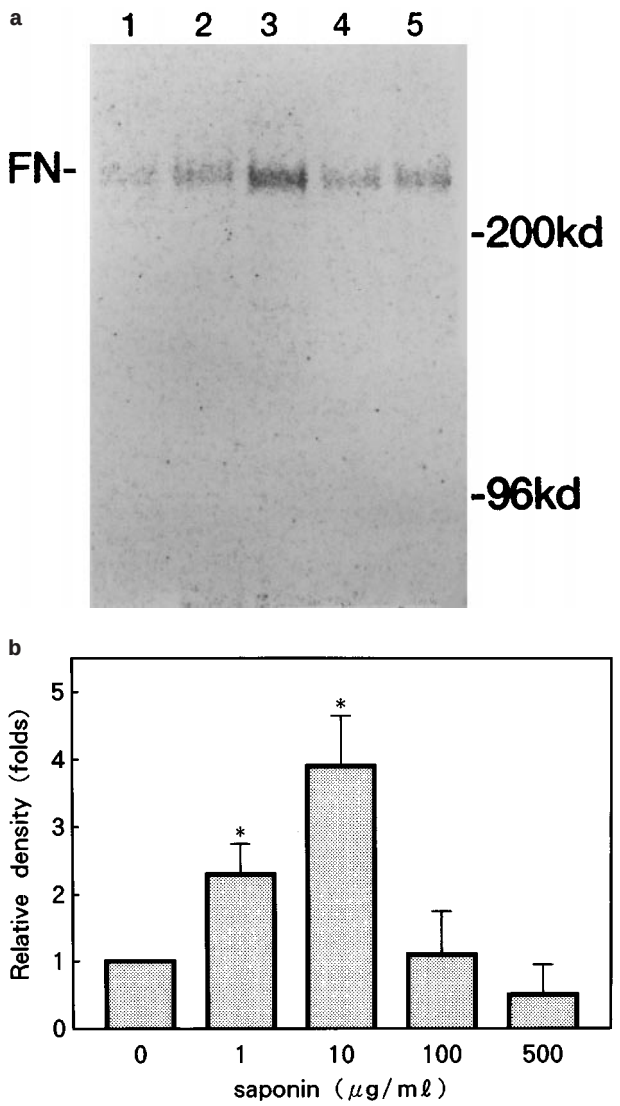

Figure 1 Effects of saponin on fibronectin synthesis in medium of fibroblasts. (a) Analysis of fibronectin in medium from fibroblasts was performed as described in Methods. Lane 1 was without saponin, lane 2 was treated with 1 μg ml^{-1} of saponin, lane 3 with 10 μg ml^{-1} lane 4 with 100 μg ml^{-1} and lane 5 with 500 μg ml^{-1}. Molecular size markers are shown on the right. FN means fibronectin bands. (b) The experiments were done with five different lots of fibroblasts and relative fibronectin synthesis was determined by densitometry. Data is represented as mean±s.d. *, $P<0.05$.

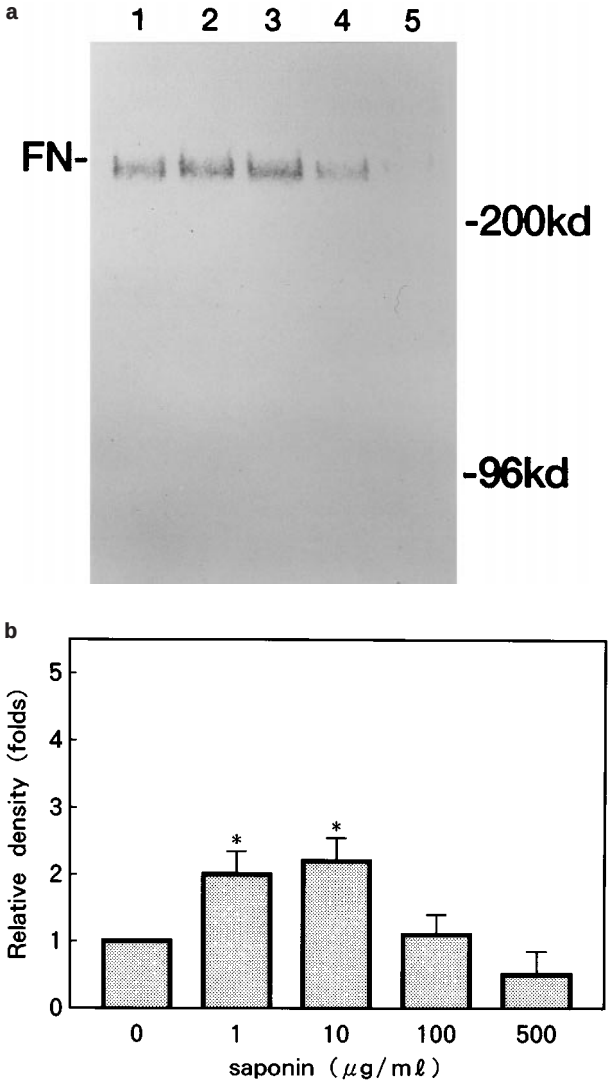

Figure 2 Effects of saponin on fibronectin synthesis in cell lysates of fibroblasts. (a) Analysis of fibronectin in cell lysates from fibroblasts was performed as described in Methods. Lane 1 was without saponin, lane 2 was treated with 1 μg ml^{-1} of saponin, lane 3 with 10 μg ml^{-1}, lane 4 with 100 μg ml^{-1} and lane 5 with 500 μg ml^{-1}. Molecular size markers are shown on the right. FN means fibronectin bands. (b) The experiments were done with five different lots of fibroblasts and relative fibronectin synthesis was determined by densitometry. Data is represented as mean±s.d. *, $P<0.05$.

Effects of saponin on TGF-β receptor expression

As the function of TGF-β1 is mediated by TGF-β receptors, cross-linking experiments were performed in order to determine the TGF-β receptor expressions in fibroblasts treated with saponin.

Three major bands, 66 kd, 90 kd and 200–300 kd, corresponded to TGF-β type I, II and III receptors, respectively (Figure 4). The intensity of the 66 kd bands in

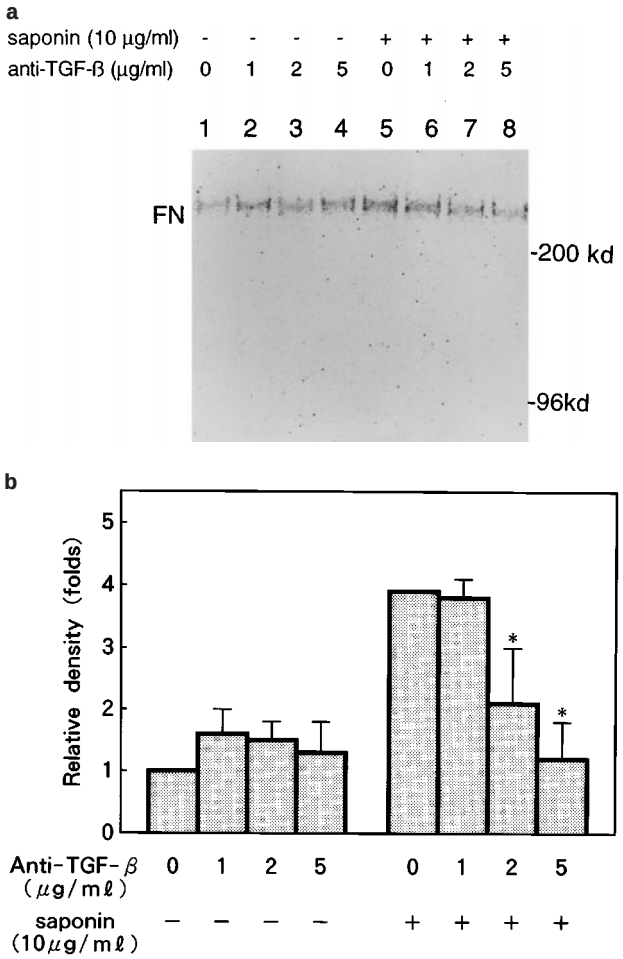

Figure 3 Effects of anti-TGF-β1 antibody on fibronectin synthesis of fibroblasts. (a) Analysis of fibronectin in medium from fibroblasts treated with various concentrations of anti-TGF-β1 antibody was performed as described in Methods. Lanes 1, 2, 3 and 4 were without saponin and lanes 5, 6, 7 and 8 with 10 μg ml^{-1} of saponin. Lanes 1 and 5 were without antibody, lanes 2 and 6 were treated with 1 μg ml^{-1}, lanes 3 and 7 with 2 μg ml^{-1}, and lanes 4 and 8 with 5 μg ml^{-1} of anti-TGF-β1 antibody. (b) The experiments were done with four different lots of fibroblasts and relative fibronectin synthesis was determined by densitometry. Data is represented as mean±s.d. *, $P<0.05$.

Table 1 Effects of saponin on TGF-β1 content in CM of fibroblasts

Saponin (μg/ml)	*Relative TGF-β1 content* (fold)
0	1
1	0.95±0.09
10	1.18±0.42
100	1.74±0.44*
250	1.87±0.06**
500	1.63±0.64

Assay of TGF-β1 content was performed as described in Methods. The experiments were done with 10 different lots of fibroblasts. Data is represented as mean±s.d. *$P<0.05$; **$P<0.01$.

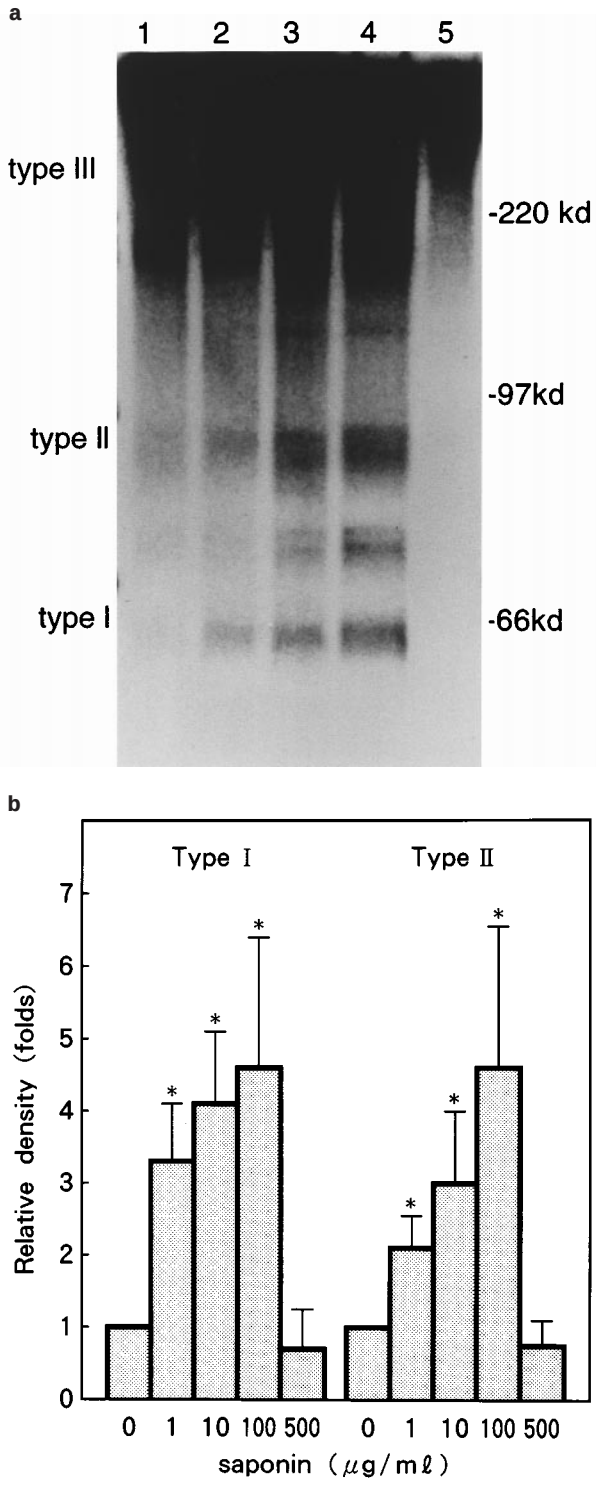

Figure 4 Binding of ^{125}I-TGF-β1 to fibroblasts treated with various concentrations of saponin. (a) Lane 1 was without saponin, lane 2 with 1 μg ml^{-1}, lane 3 with 10 μg ml^{-1}, lane 4 with 100 μg ml^{-1} and lane 5 with 500 μg ml^{-1}. Cross-linking experiments were performed as described in Methods. (b) The experiments were done with five different lots of fibroblasts and relative densities of 66 kd (TGF-β type I) and 90 kd (TGF-β type II) bands were determined by densitometry. Data is represented as mean±s.d. *, $P<0.05$.

fibroblasts treated with 1, 10, 100 and 500 μg ml^{-1} of saponin increased 3.3-, 4.1-, 4.6- and 0.7-fold, respectively, compared with that in non-treated cells (Figure 4). That of the 90 kd bands in cells treated with 1, 10, 100 and 500 μg ml^{-1} of saponin were 2.1-, 3.0-, 4.6- and 0.75-fold, respectively (Figure 4). The increases of 66 kd and 90 kd bands were statistically significant at 1, 10 and 100 μg ml^{-1} of saponin. But the intensities of the 200–300 kd bands showed no increase in cells with the addition of 1–500 μg ml^{-1} of saponin, and in fact there was a decrease in that of cells treated with 500 μg ml^{-1} of saponin. These results suggested that saponin stimulates the expression of TGF-β type I and II receptors but not that of type III receptor at the protein level in fibroblasts.

It has been reported that cross-linking experiments are able to detect TGF-β binding proteins including TGF-β receptors and other similar receptors (Franzen *et al*., 1993). To confirm that the TGF-β receptor is increased in fibroblasts by saponin, we analysed the expressions of mRNAs of TGF-β type I and II receptors.

Effects of saponin on mRNA expressions for TGF-β receptors of fibroblasts

It has been reported that the main bands of mRNAs of TGF-β type I and II receptors are both 5.5 kilo base-pairs (kb)

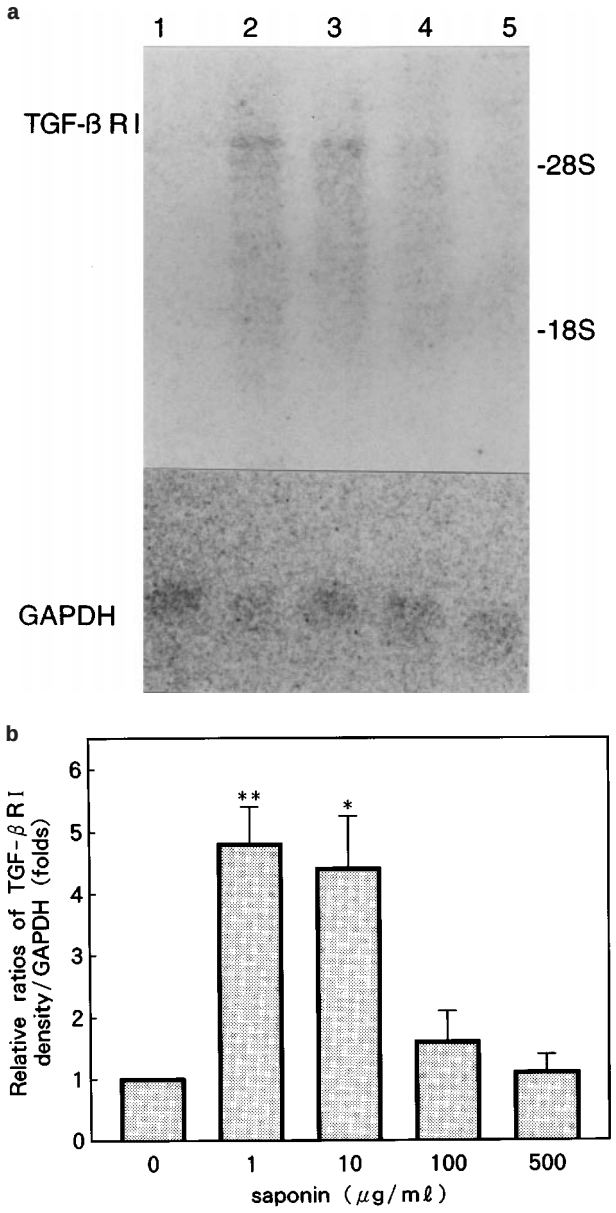

Figure 5 Effects of saponin on mRNA expression for TGF-β type I receptor of fibroblasts. (a) Lane 1 was without saponin, lane 2 with 1 μg ml^{-1}, lane 3 with 10 μg ml^{-1}, lane 4 with 100 μg ml^{-1} and lane 5 with 500 μg ml^{-1}. Northern blot analysis was performed as described in Methods using cDNA of TGF-β type I receptor or GAPDH as a probe. (b) The experiments were done with four different lots of mRNA and relative ratios of TGF-β type I receptor density/GAPDH density were determined by densitometry. Data is represented as mean$\pm$s.d. *, $P<0.05$. **, $P<0.01$.

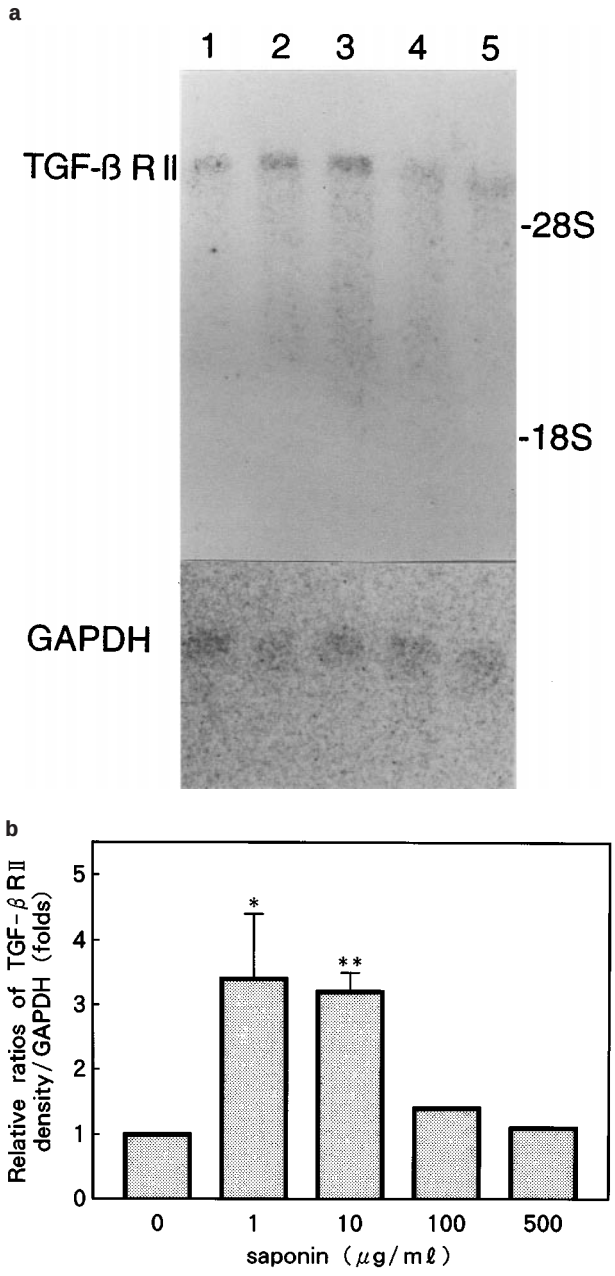

Figure 6 Effects of saponin on mRNA expression for TGF-β type II receptor of fibroblasts. (a) Lane 1 was without saponin, lane 2 with 1 μg ml^{-1}, lane 3 with 10 μg ml^{-1}, lane 4 with 100 μg ml^{-1} and lane 5 with 500 μg ml^{-1}. Northern blot analysis was performed as described in Methods using cDNA of TGF-β type II receptor or GAPDH as a probe. (b) The experiments were done with four different lots of mRNA and relative ratios of TGF-β type II receptor density/GAPDH density were determined by densitometry. Data is represented as mean$\pm$s.d. *, $P<0.05$. **, $P<0.01$.

(Franzen *et al*., 1993; Lin *et al*., 1992). The bands by Northern blot analysis using cDNAs of TGF-β type I and II receptors were detected at a size a little larger than 28S, which was about 5.5 kb. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also examined as a house-keeping gene.

The intensities of mRNAs from TGF-β type I and II receptors increased in fibroblasts treated with 1 and 10 μg ml^{-1} of saponin compared with those without treatment, but decreased with 100 and 500 μg ml^{-1} of saponin (Figures 5 and 6). The ratios of TGF-β type I receptor intensity to GAPDH were 4.8, 4.4, 1.6 and 1.1 in cells treated with 1, 10, 100 and 500 μg ml^{-1} of saponin, respectively, compared with those in non-treated cells, which were statistically significant at 1 and 10 μg ml^{-1} of saponin (Figure 5). Those of TGF-β type II receptor intensity to GAPDH were 3.4, 3.2, 1.4 and 1.1, respectively, compared with those in non-treated cells, which were statistically significant at 1 and 10 μg ml^{-1} of saponin (Figure 6). These results reflect that 1–10 μg ml^{-1} of saponin stimulates the expressions of TGF-β type I and II receptors in fibroblasts, but concentrations of 100 μg ml^{-1} or more do not.

Discussion

The present study demonstrated that saponin stimulates fibronectin synthesis of fibroblasts and modifies the expression of TGF-β receptors. Fibronectin is a large multi-functional glycoprotein containing domains that bind, on the one hand, with a number of macromolecules including collagen, fibrin, heparin and proteoglycans and, on the other, with cells *via* integrin receptors (Ruoslahti, 1988), indicating that fibronectin is involved in the interaction between fibroblasts and the extracellular matrix. Furthermore, fibronectin found in the early stage of wound healing induces the migration of fibroblasts (Ruoslahti, 1988; Colvin, 1989), suggesting that it plays a critical role in the wound healing process.

The inhibition of saponin-induced fibronectin synthesis by anti-TGF-β1 antibody suggests that its increase by saponin is mainly due to the activated TGF-β pathway. TGF-β has a variety of functions including synthesis of the extracellular matrix, growth inhibition of cells, migration of cells, differentiation and immunosuppression (Roberts & Sporn, 1990; Kanzaki *et al*., 1995). It is usually synthesized and secreted in a biologically latent form (Pircher *et al*., 1984) and activated by removal of latency-associated protein (LAP) from latent TGF-β1 (Lyons *et al*., 1988; Miyazono & Heldin, 1989; Sato & Rifkin, 1989). Current opinions suggest that TGF-β type III receptor may be indirectly involved in signal transduction as, for example, by presenting ligands to the type I and II receptors (Lopez-Casillas *et al*., 1994). TGF-β type I and II receptors have serine/threonine kinase activities and are closely related to signal tranduction of TGF-β probably by a heteromeric complex of both components (Wieser *et al*., 1993; Wrana *et al*., 1994). There are several possibilities regading the mechanism of the activation of the TGF-β pathway by saponin; (1) saponin stimulates the synthesis, secretion and activation of TGF-β1 in fibroblasts, (2) saponin changes the expressions of TGF-β receptors, and (3) the post-receptor signal transduction system is modified by saponin. Our data showed that TGF-β1 content of fibroblasts was increased by 100–250 μg ml^{-1} of saponin, higher concentrations than those for the acceleration of the fibronectin synthesis of fibroblasts. Furthermore, active TGF-β1 content was below 10% of total TGF-β1 at each concentration of saponin, indicating that TGF-β1 synthesis, secretion and activation did not contribute to the fibronectin increase in fibroblasts by saponin.

Increases of TGF-β type I and II receptors and of fibronectin synthesis were observed at the same 1–10 μg ml^{-1} of saponin at protein and mRNA levels. 100–500 μg ml^{-1} of saponin decreased TGF-β receptor expression but not GAPDH expression, suggesting that it was not a toxic effect but possibly the biphasic effect on the TGF-β receptor. Bands of TGF-β type I and II receptors examined by cross-linking were increased at 100 μg ml^{-1} of saponin compared with those without saponin, but these increases were due to TGF-β binding proteins rather than TGF-β receptors, because mRNAs of type I and II receptors were not increased at this concentration of saponin. Therefore, the change of expressions in TGF-β type I and II receptors is one of the important causes in the accelerated fibronectin synthesis of fibroblasts by saponin. As far as the post-receptor system of TGF-β is concerned, *Smad* family emphasizes the uniqueness of this signaling network (Massague, 1996; Lagna *et al*., 1996). Actually, Nakao *et al*. (1997) reported that TGF-β induces heteromeric complexes of *Smad*2, 3 and 4 and their concomitant translocation to the nucleus, which are required for efficient TGF-β signal transduction. The effect of saponin on the post-receptor signal transduction system of TGF-β remains to be elucidated.

There are several reports concerning the modification of TGF-β receptors by pathological conditions or various drugs. We previously reported that aorta and aortic smooth muscle cells (SMC) from diabetic rats expressed TGF-β type II receptor much more than those from controls, accompanied by increased fibronectin synthesis in diabetic aorta and SMC (Kanzaki *et al*. 1997). However, Chen *et al*., (1993) reported that TGF-β type II receptor is related to growth inhibition and the inhibition of phosphorylation of RB protein but not to the expression of fibronectin and plasminogen activator inhibitor-1. It has also been reported that TGF-β type II receptor is decreased in cells from atherosclerotic lesions together with an increase in extracellular matrix synthesis (McCaffrey *et al*. 1995). Frank *et al*. (1996) reported that TGF-β type I and II receptors were induced in the wound healing process, but TGF-β type II receptor expression was suppressed and type I receptor was stimulated by systemic treatment with glucocorticoids. Nakayama *et al*. (1994) reported that dexamethasone and retinoic acid (RA) enhanced gene expression of TGF-β type III receptor but did not change the expressions of TGF-β type I and II receptors in osteoblast-like cells. Taipale *et al*. (1994) reported that differentiation of human myeloid leukemia cell lines (HL-60 and U-937) was induced by phorbol 12-myristate 13-acetate, tumor necrosis factor alpha and RA, accompanied by increases in TGF-β type I and II receptor expressions. Cohen *et al*. (1995) reported that RA increased the expressions of TGF-β type I, II and III receptors, together with growth arrest and induction of differentiation in RA sensitive human neuroblastoma cell lines. From these various reports, it is difficult to determine which receptor is finally responsible for the specific function of TGF-β, and also the effects of drugs on TGF-β receptors are variable in different types of cells. It is may be supposed that the post-receptor system corresponds to the respective function of TGF-β.

Saponin, the active component of Ginseng Radix rubra, consists of many ginsenosides. It is unclear just which component(s) is particularly responsible for the stimulation of fibronectin synthesis and changes of TGF-β receptor expressions of fibroblasts. The major ginsenosides of saponin are a group of dammarane saponins which have a similar structure to cholesterol and steroid hormones (Yamamoto 1988).

Glucocorticoids, one of the steroid hormones, have been reported to change the expressions of TGF-β type I and II receptors (Frank *et al*., 1996). Therefore, ginsenosides resembling sterols by their structure may be responsible for changes of TGF-β receptor expressions in fibroblasts.

In summary, saponin stimulates the wound healing process through changes of the extracellular matrix metabolism, accompanied by modification of TGF-β receptor expressions in fibroblasts.

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