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Type I Collagen Inhibits Hydroxyl Radical–Induced Apoptosis

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The extracellular matrix (ECM) plays an important role in cell differentiation and apoptosis. Collagen is the major component of ECM. Here, an ESR signal of the hydroxyl radicals (•OH) generated via Fe^{2+}-mediated Fenton reaction was found to be significantly inhibited by type I collagen. Further study showed that type I collagen also inhibited cell apoptosis induced by •OH, as evidenced by morphological criteria (DAPI and annexin V staining) and quantitive assays for apoptotic cells (MTT and flow-cytometric assay for subG1 cells). By addition of type I collagen in HeLa cells, the lipid peroxidation caused by •OH was inhibited and the cellular GSH was protected. In comparison with type I collagen, BSA and the denatured collagen, gelatin, lacked such antioxidative and antiapoptotic effects. Together, the results suggest that type I collagen can uniquely prevent •OH-mediated apoptosis by scavenging free radicals.

Key words: apoptosis, electron spin resonance spectroscopy, hydroxyl radicals, type I collagen.

The extracellular matrix (ECM) is a dynamic and complex source of molecular information that profoundly regulates cell behavior. Like a variety of cytokines, growth factors and hormones, the ECM plays a key role in cell differentiation, growth, and migration (*1*). Recent reports have implied that ECM also can influence survival and apoptosis of several cell lineages, including glomerular mesangial cells and fibroblasts. Many cell types undergo cell death when deprived of adhesion to the appropriate ECM. For example, endothelial and epithelial cells die by apoptosis when detached from substrate with Arg-Gly-Asp (RGD) peptides or when the attachment is prevented by growing them in suspension (*2, 3*). Basement membranes are thin layers of a specialized ECM that provide the supporting structure on which epithelial and endothelial cells grow (*4*). Some reports indicated that basement membranes were the potent mediator of differentiation (*5*) and cell cycle progression (*6*), as well as quiescence in the normal tissue (*7*). Boudreau *et al.* (*8*) reported that the extracellular matrix of basement membranes induced differentiation and suppressed apoptosis in mammary epithelial cells, whereas cells lacking basement membranes lost their differentiated phenotype and underwent apoptosis. Makino *et al.* (*9*) have also shown that basement membranes matrix prevented cultured mesangial cells from undergoing apoptosis after serum deprivation, thus promoting their survival. Thus the results suggest that the ECM must play a central role in coordinating expression of both positive and negative regulators of the cell cycle and thus protect differentiated cells from undergoing apoptosis.

Collagen is an essential component of ECM in organisms. It is reasonable to consider that collagen may also have anti-apoptosis functions for a component of ECM. Collagen has been shown to regulate cell adhesion, morphology, migration, viability, growth, and differentiation besides its mechanical structure function (*10–12*). Many kinds of diseases, such as silicosis, liver cirrhosis, diabetes, and some genetic diseases are related to the pathological changes of collagen (*13, 14*). Several other reports have shown that collagen could suppress apoptosis induced by deprived serum (*15, 16*). While much is known about regulation of apoptosis by growth factors, cytokines, and hormones, the role of collagen in regulating apoptosis, although well recognized, is still little understood. This paper focuses on the role of collagen in regulating free radical-induced apoptosis.

Both endogenous and environmental free radicals are very important agents or factors that induce cell apoptosis and various diseases, including several collagen diseases and lung or liver fibrosis (*17, 18*). However, there also exist self-defense systems against free radical, including high-molecular-weight antioxidants such as superoxide dismutase (SOD), catalase and GSH-Px and low-molecular-weight antioxidants such as vitamins C, E, and A and polyphenols. ECM is another ubiquitous self-defense system against oxidation stress. The relationship between ECM and free radicals and the role of ECM in protecting cells from apoptosis, particularly that induced by free radicals is of interest.

Free radicals play an important role in the synthesis of collagen. Evidence shows that reactive oxygen species (ROS) could increase the ECM gene expression in cultured human mesangial cells (*19*). However, Arisawa *et al.* (*20*) suggested that collagen was denatured by scavenging hydroxyl radical to protect fibroblasts from damage, so the remodeling of collagen may be influenced by radicals.

In previous papers (*21, 22*), the authors reported on the hydroxyl radical (•OH)–induced apoptotic pathway in hu-

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man tumor cells, which provides an appropriate system to investigate the role of collagen in inhibiting apoptosis induced by ROS. The current paper describes the unique effect of type I collagen in protecting cells from apoptosis by scavenging •OH.

MATERIALS AND METHODS

Materials—Type I collagen from rat tail, glutathione (GSH), 4',6'-diamidin-2-phenylindole (DAPI) and propidium iodide (PI) were purchased from Sigma (St. Louis, MO, USA). 1,4-Dithiothreitol (DTT) was purchased from Promega (Madison, WI, USA). Annexin V assay kit was purchased from Clontech (Palo Alto, CA, USA). All other substances were from commercial sources and of analytical grade.

Cell Culture—HeLa cells were cultured in Dulbecco's modified Eagle's medium with high glucose (4.5 g/l) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% fetal calf serum under standardized conditions (37°C, 5% CO_2) in a humidified atmosphere. Exponentially growing cells were used for experiments.

Induction of Apoptosis and Experimental Treatments—Exponentially growing cells were treated with Fenton reaction mixture containing 0.1 mM $FeSO_4$ and 0.6 mM H_2O_2 for the indicated length of time (from 0 to 24 h) to induce apoptosis (apoptosis-positive control). The continuous generation of •OH for more than 30 min was confirmed by the ESR spin trapping technique (see Fig. 1b). To study the effect of type I collagen on the •OH-induced apoptosis, 50-300 μg/ml type I collagen was added into culture media 2 h before the treatment with Fenton reaction mixture (collagen-treatment group). Four control experiments were performed, either without pretreatment (normal control), or by pretreatment with BSA, gelatin or GSH instead of collagen.

ESR Assay for •OH Radicals—•OH radicals, generated via the Fe^{2+}-mediated Fenton reaction (*23*), were detected by spin-trap electron spin resonance spectroscopy (ESR). Briefly, 0.1 mM $FeSO_4$ was mixed with 0.6 mM H_2O_2 in the presence of 50 mM DMPO, an •OH radical trap reagent, and then the Fenton reaction mixtures were sucked into a silica capillary sample tube. ESR spectrum scanning was carried out at frequency modulation of 100 KH, amplitude modulation of 2G, sp = dB10, with a field of 328 ± 10 mT using an ER200/D ESR spectroscope (Bruker, German). Four ESR peaks for •OH radicals were observed. To evaluate the effect of type I collagen on each of the •OH radicals, the time course curves of the second peak of the •OH ESR spectrum were recorded for 30 min. In the other four experimental systems, the Fenton reaction mixture contained 150 μg/ml type I collagen, gelatin, or BSA or 1 mM GSH. HeLa cells (1×10^6) were incubated with 150 μg/ml type I collagen for 2 h and treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 for 10 min, then ESR signal was immediately recorded after addition of 100 mM DMPO to the reaction mixture.

Lipid Peroxidation Measurement—The extent of lipid peroxidation in HeLa cells was estimated by measurement of malondialdehyde (MDA) formation using the thiobarbituric acid method. After treatment, cells were collected and washed twice with ice-cold D-Hanks solution. Then the MDA was measured and calculated according to the method of Nelson *et al.* (*24*). Protein concentration was determined by the Bradford method.

Determination of Intracellular GSH Content—Intracellular GSH was dethermined as described previously (*25*). The concentration of intracellular GSH was expressed as nanomoles per mg protein.

Colorimetric MTT Assay—Cell viability was determined by the tetrazolium dye colorimetric test (MTT test) (*26*). The MTT absorbance was read on a plate reader (Bio-Rad, model 3550, CA, USA) using a test wavelength of 550 nm and a reference wavelength of 630 nm.

DAPI Staining—Cells growing on glass coverslips, precoated with 1 mg/ml of poly-L-lysine (Sigma, Mr 3.7×10^4) at the density of 3×10^4 cells/cm^2, were rinsed in D-Hanks solution and fixed with 2% methanol. Cells were stained with 1 μg/ml DAPI in PBS for 30 min. Apoptotic cells were determined by evaluating nuclear morphology using a fluorescence microscope (Nikon Fluophot).

Annexin V Staining and FACS (Fluorescence-Activated Cell Sorter) Analysis—The annexin V method was used to evaluate the number of cells undergoing apoptosis (*27*). For fluorescence microscopy, cells growing on glass coverlips were rinsed and fixed as above, then apoptotic cells were stained by fluorescence dyes using an annexin V-FITC/propidium iodide (PI) kit according to the manufacturer's instructions. The stained cells were observed under a fluorescence microscope (Nikon Fluophot). For quantitative FACS analysis of the apoptotic cells, trypsinized cells were washed in ice-cold D-Hanks solution and also stained with a fluorescence annexin V-FITC/PI kit. The fluorescence intensity of apoptotic cells was measured and analyzed using a Coulter Elite Flow Cytometer.

Flow Cytometry for Sub-G1 Analysis—After trypsinization, cells were adjusted to the same cell number (~1×10^6), washed with 10 ml of PBS, fixed in 70% ethanol for 30 min at −20°C, and suspended in 500 μl of PBS containing RNase A (20 mg/ml) for 30 min at 37°C. Fixed cells were stained in a final volume of 1 ml with propidium iodide (50 ng/liter), then subjected to FACS analysis as described (*28*).

Statistical Analysis—Group data are expressed as the means ± SE. Significance was assessed by either a two tailed Students *t*-test or a one-way analysis of variance (ANOVA). All data represent the average of at least three independent experiments.

RESULTS

Type I Collagen Inhibited •OH-Generation by the Fenton Reaction—•OH radicals were generated *via* a Fe^{2+}-mediated Fenton reaction according to the protocol in "MATERIALS AND METHODS" and detected by spin-trap ESR. The specific ESR spectrum for •OH radical signals consisted of four peaks within the field of 328 ± 10 mT (Fig. 1a). However, in the presence of soluble type I collagen (150 μg/ml), the four characteristic ESR peaks for •OH radicals almost disappeared, whereas the addition of BSA or gelatin (150 μg/ml) did not significantly influence the signal intensity of ESR spectra (data not shown). To evaluate quantitatively the inhibitory effect of collagen on •OH-generation, the time-course curves of the second peak of the •OH ESR spectrum were recorded for 30 min (Fig. 1b). The second ESR peak of •OH was sustained longer than 30 min, although it did undergo a gradual weakening. GSH, a biological free

radical scavenger, significantly inhibited the •OH –generation over an extended period of time; and type I collagen similarly inhibited the •OH generation, the ESR signal of •OH being even a little weaker than that in the presence of GSH in the early period of reaction. On the other hand, BSA and gelatin (a known denatured collagen) did not evidently reduce the amplitude of ESR signals of •OH with time. These ESR results demonstrated that nondenatured type I collagen can inhibit the generation or maintenance of •OH in a Fenton reaction system.

Type I Collagen Inhibited Lipid Peroxidation—•OH can cause lipid peroxidation as it reacts with the polyunsaturated fat on the membrane (*29*). MDA is the product of membrane lipid peroxidation caused by ROS. The MDA content was analysed to assess the level of lipid peroxidation in HeLa cells after treatment with •OH. As shown in Fig. 2, the •OH treatment (0.1 mM $FeSO_4$/0.6 mM H_2O_2) caused more than a threefold increase of MDA, which occurs in a dose-dependent manner (data not shown). Pretreatment of cells with type I collagen effectively reduced the formation of MDA by •OH radicals ($p < 0.001$).

Type I Collagen Inhibited the Reduction of Cellular GSH Content Level Caused by •OH Radicals—Glutathione (GSH) measurement was used to evaluate the intracellular redox status (Fig. 3). Cellular GSH is an important compound that functions as a substrate or cofactor of protective enzymes and as an efficient radical scavenger. Its concentration is an index of the intracellular redox status. In this study, in the presence of the Fe^{2+}-H_2O_2 system, a decrease of 88.4% was observed in the intracellular total GSH content as compared with untreated cells ($p < 0.01$). However, in the presence of type I collagen the GSH content in •OH-treated cells increased approximately threefold, compared with the •OH treatment group without collagen. This suggested that type I collagen could prevent the changes of intracellular redox status caused by •OH.

Type I Collagen Inhibits Apoptosis in HeLa Cells Induced by Exogenous •OH—Since collagen can inhibit •OH generation, it is reasonable to consider that type I collagen may

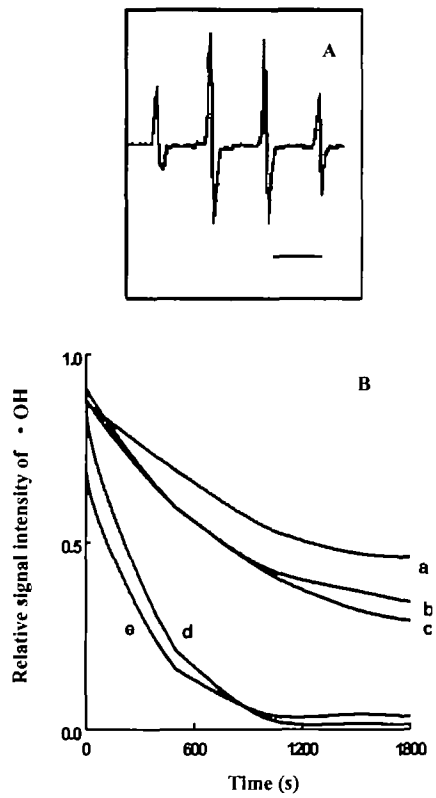

Fig. 1. **Effect of type I collagen, BSA, gelatin and GSH on •OH generation.** A: ESR signals of •OH generated via Fenton reaction. The Fenton reaction mixture contained 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and 50 mM DMPO. Bar, 20 Gauss. B: Attenuation curves of the second ESR peak of •OH. The reaction mixtures contained 0.1 mM $FeSO_4$/0.6 mM H_2O_2, 50 mM DMPO plus 150 μg/ml BSA (b), 150 μg/ml gelatin (c), 1 mM GSH (d), or 150 μg/ml type I collagen (e). (a) Control (without additional supplements). The measurements were carried out continuously for 30 min. All *in vitro* experiments were done in triplicate.

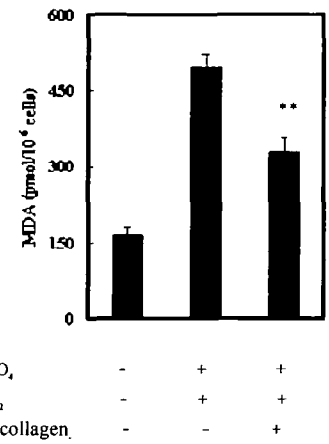

Fig. 2. **Effect of type I collagen on •OH-induced lipid peroxidation in HeLa cells.** Levels of MDA were quantified after treatment with •OH or the combination of •OH and type I collagen for 24 h. For the collagen-treatment group, HeLa cells were pretreated with type I collagen for 2 h before addition of 0.1 mM $FeSO_4$/0.6 mM H_2O_2. Values are the means of three independent experiments. Error bars indicate standard deviations (**$p < 0.01$ *versus* •OH-treated group).

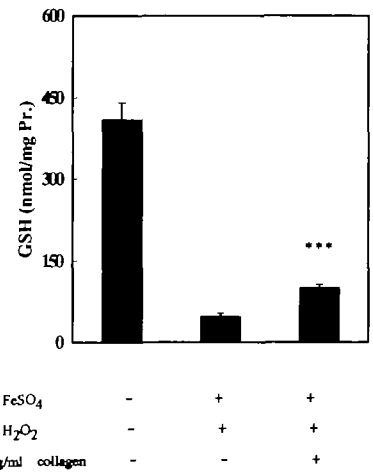

Fig. 3. **Effect of type I collagen on intracellular GSH in HeLa cells after treatment with •OH for 24 h.** For the collagen-treatment group, HeLa cells were pretreated with type I collagen for 2 h before addition of 0.1 mM $FeSO_4$/0.6 mM H_2O_2. Values are the means of three independent experiments. Error bars indicate standard deviations (**$p < 0.001$ *versus* •OH-treated group).

inhibit apoptosis by scavenging free radicals. In the present work, apoptosis of HeLa cells was induced by •OH generated via the Fe^{2+}-mediated Fenton reaction as the experimental apoptotic model, and the effects of type I collagen in portecting cells from apoptosis were confirmed on the basis of the following experiments.

MTT Assay—Firstly, a MTT assay was used to analyze the effect of type I collagen on the viability of cells treated with •OH. Viable HeLa cells decreased significantly with the increase of •OH concentration (data not shown), whereas the cell death induced by •OH was significantly inhibited by pretreatment with type I collagen for 2 h in a dose-dependent manner, concomitant with the decrease of •OH concentration (Fig. 4a). As shown in Fig. 4b, compared with type I collagen (150 μg/ml), both BSA and gelatin did not inhibit the •OH-induced HeLa cell death. As a positive control, GSH, a known scavenger of •OH, showed a significant inhibitory effect.

DAPI Staining—Initially, cells in each group were adherent and spread on coverslips to an equal extent (Fig. 5A). However, •OH-treated cells detached after 24 h, and the morphological features of apoptosis (*i.e.*, cell shrinkage and nuclear condensation) were evident in DAPI-stained cells showing disintegration in the nuclei. This appeared in approximately 65% of the cells treated with •OH (Fig. 5B), contrasting with less than 30% of cells pretreated with type I collagen (Fig. 5C). However, cells pretreated with 150 μg/ml type I collagen remained viable and well spread after 24 h of •OH treatment.

Annexin V Assay–The apoptotic cells were identified by the binding of annexin V to the cell surface as observed by fluorescence microscopy (Fig. 6A) and flow cytometric assay (Fig. 6B). Annexin V is an anticoagulant protein that preferentially binds to negatively charged phospholipids and can be used to identify cells in the early stages of apoptosis. Propidium iodide enters cells with membranes disrupted by necrosis or in the late stages of apoptosis. Therefore, conjugated annexin V/FITC–staining (green fluorescence) and propidium iodide (red fluorescence) were used after 24 h of oxidative stress to identify early apoptosis and cell death. Both results in Fig. 4 confirmed the anti-apoptotic effect of type I collagen. Few cells in the control group (nontreated with •OH) were stained (Fig. 6Aa), whereas a majority of •OH-treated cells displayed green and red staining (Fig. 6Ab), and positive staining was markedly reduced in the •OH plus collagen treatment group (Fig. 6Ac). Flow cytometric assay (Fig. 6B) showed that less than 34% of cells treated with collagen (150 μg/ml) could be identified as apoptotic, compared with the 68.7% of apoptotic cells found in the absence of collagen.

Sub G1 Assay—SubG1 peak in the FACS chart represents the percentage of apoptotic cells with a DNA content of less than 2 N (subG1 DNA). The subG1 assay (Fig. 7) demonstrated approximately 69.1% of subG1 cells in the •OH-treatment group, The apoptosis-inhibiting efficiency of soluble collagen (150 μg/ml) in the medium (~35% of subG1 cells) was equal to 62% of potential of 1 mM GSH (13% of subG1 cells). Pre-coating type I collagen on the bottom surface raised the survival proportion of •OH-treated cells by 1.75 times, although its efficiency was lower than supplying soluble collagen into the medium. In contrast, neither BSA nor gelatin reduced the high percentage of subG1 cells induced by •OH-treatment. These results further proved the significant antiapoptotic potential of nondenatured collagen, which is quite different from BSA and gelatin, and confirmed the significant inhibitory effect of collagen on apoptosis induced by •OH generation.

DISCUSSION

The present work makes it clear that type I collagen possesses two closely related functions: inhibition of •OH-generation, and significant protection of cells from apoptosis induced by the Fe^{2+}-mediated Fenton reaction. It is reasonable to propose that the former could be the direct and primary basis of the later. Therefore, in the presence of type I collagen, MDA (an oxidated production of plasma-membrane lipids by ROS) was significantly reduced, and cytoplasmic GSH antioxidative activity was significantly increased.

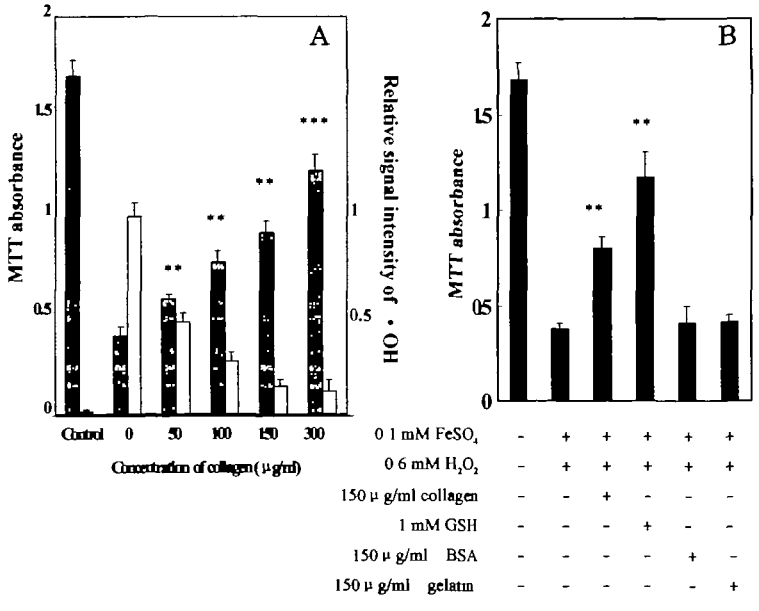

Fig. 4. **Type I collagen inhibits •OH-induced cell death in HeLa cells measured by the MTT test.** A: HeLa cells were pretreated with different concentrations of type I collagen for 2 h, then treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2. After incubation for 10 min, the •OH concentration in the culture medium of HeLa cells (1×10^6) was measured by ESR with DMPO (100 mM) as the spin trap. The MTT activity of HeLa cells was assayed after incubation for 24 h. The results of MTT assay (black bar) and the •OH level (white bar) for different concentrations of type I collagen are shown in parallel. Control: without 0.1 mM $FeSO_4$/0.6 mM H_2O_2 treatment. B: HeLa cells were pretreated with type I collagen, GSH, BSA and gelatin for 2 h, then treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and incubated for 24 h. Values are the means of three independent experiments. Error bars indicate standard deviations ($^{**}p < 0.01$, $^{***}p < 0.001$ *versus* •OH-treated group).

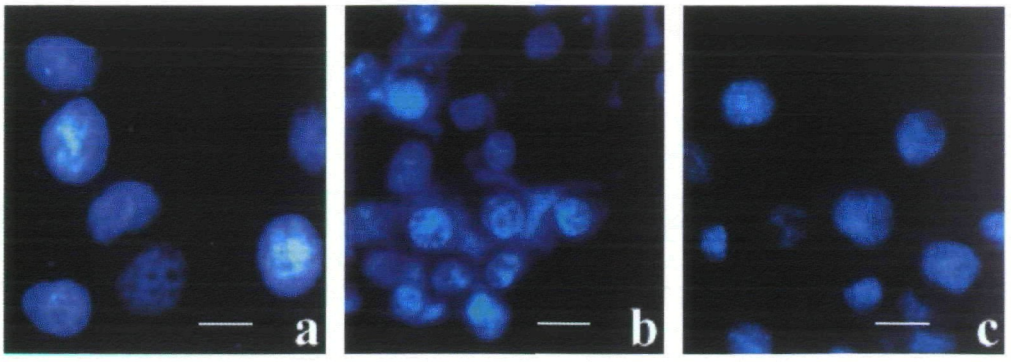

Fig. 5. **Type I collagen prevented •OH induced-apoptosis in HeLa cells.** Apoptosis was detected by DAPI staining. (a) Normal control without •OH treatment (b) Apoptosis-positive control; HeLa cells were treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and incubated for 24 h. (c) Collagen-pretreatment group; HeLa cells were pretreated with type I collagen for 2 h, then treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and incubated for 24 h. Bar, 11 μm.

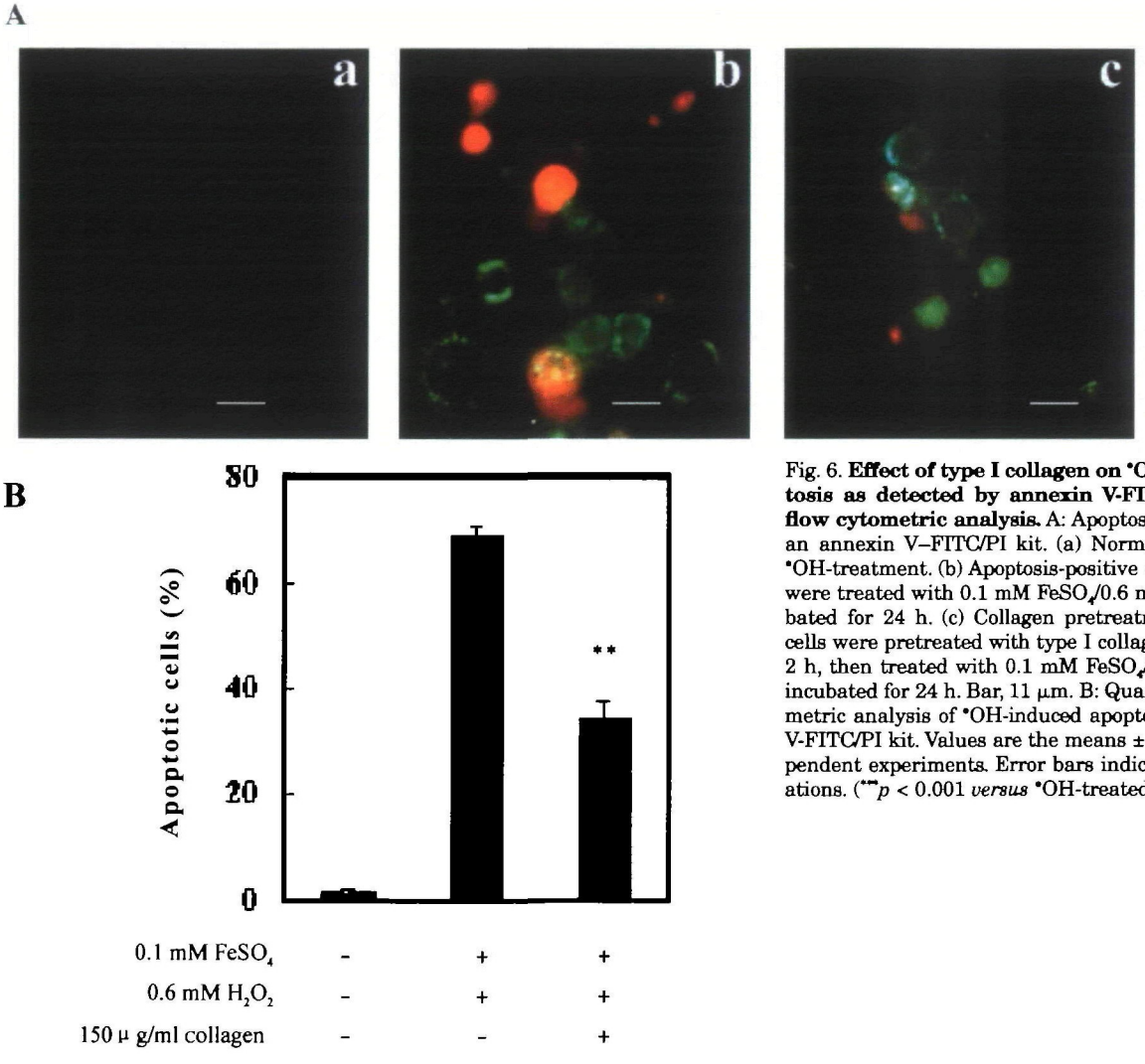

Fig. 6. **Effect of type I collagen on •OH-induced apoptosis as detected by annexin V-FITC/PI assay and flow cytometric analysis.** A: Apoptosis was detected by an annexin V–FITC/PI kit. (a) Normal control without •OH-treatment. (b) Apoptosis-positive control; HeLa cells were treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and incubated for 24 h. (c) Collagen pretreatment group, HeLa cells were pretreated with type I collagen (150 μg/ml) for 2 h, then treated with 0.1 mM $FeSO_4$/0.6 mM H_2O_2 and incubated for 24 h. Bar, 11 μm. B: Quantitative flow cytometric analysis of •OH-induced apoptosis using annexin V-FITC/PI kit. Values are the means ± S.D. of three independent experiments. Error bars indicate standard deviations. (**$p < 0.001$ *versus* •OH-treated group)

However, it is possible that other mechanisms besides the direct antioxidant pathway may be involved in the inhibitory effects of collagen on the apoptosis induced by ROS. In this work, another experiment protocol was designed in which soluble type I collagen was added to culture dishes for 1–2 h, then the collagen solution was removed, and after another 1–2 h a layer of collagen gel membrane formed on the bottom of the dishes. Confluent cells grown on the collagen-coated dishes also resisted the attack of •OH radicals more strongly than those grown in the absence of collagen, although the soluble collagen added to the medium was approximately 1.3 times more efficient in preventing •OH-induced apoptosis than the collagen coated dishes (Fig. 7). For the cells growing on collagen-coated dishes, collagen exerted other actions rather than its action as a ROS scavenger. Hoyt *et al.* (*30*) reported

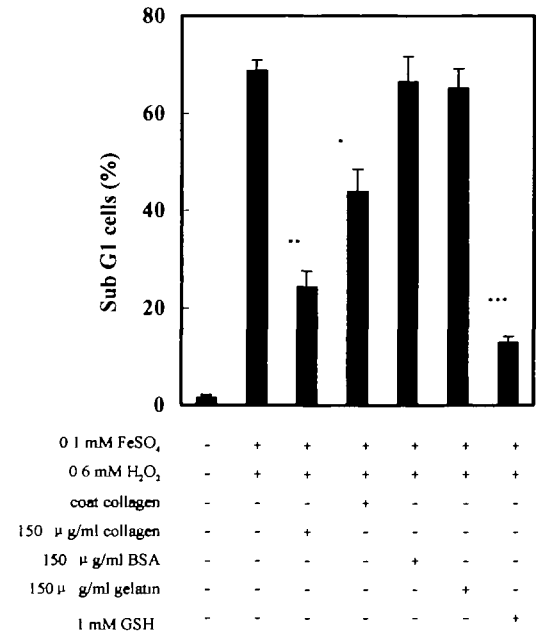

Fig. 7. **Comparison of antiapoptotic efficency of collagen, BSA, gelatin, and GSH.** The apoptotic cells were detected by flow-cytometric analysis. Values are the means ± S.D. of three independent experiments. Error bars indicate standard deviations. (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ *versus* •OH-treated group).

that collagen was a survival factor against LPS-induced apoptosis in the cultured endothelial cells. It was proposed that the beta (1)-integrin–collagen interaction played a key role in inhibition of apoptosis in chondrocytes (*31*), and type I collagen could reassemble as a pericellular matrix network, resulting in the increase of interaction between collagen and its cell receptors. Such interaction can enhance cell spreading, which is available for cell survival and proliferation.

In the present work, the Fe^{2+}-mediated Fenton reaction and sequential apoptotic events occurred in cultural media supplemented with 10% FCS, which contains a variety of plasma proteins. The most abundant bovine plasma protein, BSA, did not display obvious antioxidant and antiapoptotic properties. However, type I collagen displayed these functions prominently. It not only sequestered the •OH radicals generated by the Fenton reaction, but also inhibited sequential apoptosis. Moreover, the data indicated that only the non-denatured collagen possessed such unique properties, whereas denatured collagen, gelatin, lacked both antioxidative and antiapoptotic functions. A similar result was observed in a kininogen-induced apoptosis system, in which collagen can inhibit the kininogen-induced apoptosis but gelatin can not (Jing-Chuan Zhang *et al.*, unpublished communication). These results implied that a perfect molecular structure was required for the unique antioxidative function of collagen, though little is known about its molecular mechanisms. Hawkins *et al.* (*32*) indicated that the interaction of collagen with hydroxyl radicals occurred at a specific site, such as a double-sided chain (•CHR'R"), and α-carbon[•C(R)(NH–)CO-, R = side-chain] radicals. Sajithlal GB *et al.* (*33*) reported that the formation of advanced glycation end-products (AGEs) was involved in the oxidation of collagen. In preparation of gelatin, the triple helix structure of collagen is lost, and the side chains of some amino acid residues and sugar chains are also damaged. These molecular structural changes may destroy the electrodynamical stable configuration conducive to ROS trapping, for example, causing the loss of the Fe^{2+}-binding ability of collagen, and damage or cleavage of the side chain of amino acid residues and sugar chains with site-specific activity.

Recently, the oxidative modification and damage of collagen has received considerable attention. The relationship of oxidative damage to collagen with several pathological processes, such as rheumatoid arthritis and diabetes, has also been recognized. Close attention was given to the interaction of collagen with ROS, which posed an important question regarding the role of collagen as a sink for ROS. The ESR spectrum results clearly showed that type I collagen was able to directly quench the •OH radicals generated by the Fenton reaction. We found that addition of 250 μg/ml of type I collagen to the reaction system sequestered the hydroxyl radicals in several seconds. Though the ESR spectra were recorded immediately after the mixture was prepared, which process took only 6–7 seconds, the •OH radical signal was nearly zero from the beginning. At the lower concentration of 150 μg/ml of type I collagen, the relative signal intensity (approximately 0.7) at the start of analysis could be recorded, but it was still lower than that with GSH, and it decreased rapidly. These results suggest that there are various differences between collagen and GSH in their inhibitory effects on the hydroxyl radical. GSH is widely accepted as a free radical scavenger and its mechanism of thoroughly scavenging the free radicals is clearly understood. But the mechanism by which type I collagen acts as a sink for hydroxyl radicals is still unknown. Collagen may interact with the hydroxyl radical indirectly rather than directly as a hydroxyl radical scavenger. This interaction might even produce a less toxic intermediate adduct so that the collagen can not as completely inhibit the hydroxyl radical as GSH. Since collagen is an important ECM constituent in the majority of tissues, collagen could function as a ubiquitous antioxidant and form a huge "physiologic buffer" against the oxidative stress to protect cells from apoptosis and aging.

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