Protective Effect of Transfection with Secretable Superoxide Dismutase (SOD) (a Signal Sequence-SOD Fusion Protein Coding cDNA) Expression Vector on Superoxide Anion-Induced Cytotoxicity in Vitro

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For ex vivo gene therapy, superoxide dismutase (SOD) must be secreted into the extracellular space and delivered to damaged cells. Recombinant DNA technique can be used to produce a secretory protein that is fused to a non-secretory protein and a signal peptide of another secretory protein gene. We constructed a secretable SOD eukaryotic expression vector which expresses human SOD cDNA by fusing it to the signal peptide DNA sequence of the human interleukin-2 (IL-2) gene. The ILSOD cDNA constructed by PCR-based gene expression was ligated into the multicloning site of the pRC/CMV plasmid (pRC/CMV-ILSOD). Rat lung epithelial like cells (L2 cells) were transfected with pRC/CMV-ILSOD by lipofection. The extracellular SOD activity of ILSOD-L2 cells (transfected cells with pRC/CMV-ILSOD) was 3 times as high as that of host cells. We used the xanthin (X)/xanthine oxidase (XO) system to produce superoxide anions at the extracellular space. We initially investigated the direct cytotoxicity of superoxide anions upon cells. Host and ILSOD-L2 cells were killed by using X/XO, although the sensitivity of the ILSOD-L2 cells to X/XO induced cytotoxicity was significantly decreased compared with that of host cells. The production of lipid peroxidated substances in the host in the presence of X/XO increased to about twice the control (absence of X/XO) level. However, that of ILSOD-L2 cells did not change in the presence of X/XO. Therefore, ILSOD-L2 cells were resistant to X/XO induced lipid peroxidation. These findings indicated that the SOD gene transfection protected against direct oxidant stress by X/XO.

We then investigated the effect of extracellular SOD secreted from ILSOD-L2 cells on extracellular superoxide anion induced cytotoxicity in normal cells. The conditioned media of host cells had no significant effect upon X/XO induced cytotoxicity. However, the conditioned media of ILSOD-L2 cells protected against X/XO induced cytotoxicity. Furthermore, the conditioned medium of ILSOD-L2 cells was more effective than that of host cells against the production of lipid peroxidated substances by normal cells under conditions of oxidative stress. These results indicated that non-secretable protein could be delivered to target cells by means of DNA engineering. This strategy could thus provide an ex vivo means of applying gene therapy using non-secretable proteins.

Key words gene therapy; superoxide dismutase; interleukin-2; fusion gene; secretable superoxide dismutase; cytotoxicity

Severe lung diseases such as paraquat toxicity and adult respiratory distress syndrome are closely related to pulmonary oxygen toxicity.1–4 However, the clinical application of superoxide dismutase (SOD) is governed by several parameters, including half life in the body, organ specificity and intracellular penetration.5 SOD is rapidly eliminated within a few minutes by catabolism in the kidney.6 Furthermore, the intravenous and subcutaneous administration of SOD or liposome SOD dose not improve these diseases, because of the difficulties of delivering SOD to the lung.7,8 To solve this problem, we proposed gene therapy as a new approach to delivering SOD.9 We established a eukaryotic SOD expression plasmid in which human SOD (hSOD) cDNA is inserted into eukaryotic expression plasmids (pRC/CMV and pRC/RSV) under the control of the cytomegalovirus and Raus Sarcoma virus promoters.10–12 We transfected these hSOD expression plasmids into cultured rat lung and skin cells by lipofection. The intracellular SOD activities in these transformed cells were about twice as high as those in the host cells, whereas the extracellular SOD activities were similar. Furthermore, the SOD activities in transformed cells were enhanced for 60 d after selecting clones. Under paraquat or xanthine (X)/xanthine oxidase (XO) treatment to generate active oxygen species in intracellular or extracellular spaces, significantly more transformed than host cells survived. Lipid peroxidation in the host cells significantly increased in the presence of paraquat or X/XO. However, the production of lipid peroxidation in the transformed cells did not change under either condition. Thus, the transfected SOD gene protected against paraquat and X/XO-induced cytotoxicity. These findings showed that the increased level of intracellular SOD activity protected against both intracellular (paraquat) and extracellular (X/XO) superoxide anion stress and indicated that in vivo gene therapy could be applied using the SOD gene.

Most protocols for gene therapy require the genetic modification of autologous cells from patients. For somatic gene therapy ex vivo, SOD must be secreted into the extracellular space and delivered to damaged cells. Various secretory proteins are synthesized as precursors with a signal peptide consisting of 20 to 40 hydrophobic amino acids.13 Several hybrid proteins have been generated by means of recombinant procedures.14,15 This technique can fuse a secretable protein with a non-secretable protein and the signal peptide of another secretable protein gene. We designed an expression vector

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(pRc/CMV-ILSOD) for secretable SOD protein. The pRc/CMV-ILSOD plasmid was constructed from a eukaryotic expression vector (pRc/CMV) and the ILSOD cDNA, which consisted of a hSOD cDNA fused to the signal peptide sequence of the interleukin-2 (IL-2) gene. Sasada et al. have indicated that not only the signal peptide, but also a specific amino acid sequence and/or a conformation of polypeptides suitable for the signal peptide is necessary for the efficient secretion of a foreign protein. Therefore, the ILSOD cDNA included a DNA sequence encoding 11 N-terminal amino acid residues of IL-2 between the IL-2 signal peptide sequence gene and SOD cDNA. ILSOD cDNA was inserted into a eukaryotic expression plasmid (pRc/CMV) under the control of the cytomegalovirus promoter, then transfected into cultured rat lung cells by means of lipofection. Here, we report the expression of hSOD cDNA by fusing it to a signal peptide sequences of the IL-2 gene. We also describe the effect of transfection with a vector encoding the signal peptide of the IL-2-SOD fusion gene on X/XO-induced cytotoxicity in cultured rat lung cells.

Transfecting cultured cells with plasmid DNA in vitro is a useful model for understanding expression. This model can be used in animal studies and clinical trials.

MATERIALS AND METHODS

Materials Human Cu, Zn-SOD cDNA (hSOD cDNA) and recombinant human Cu, Zn-SOD (3200 U/mg protein) were supplied by Asahi Chemical Industries (Tokyo, Japan). The DNA encoding the human IL-2 signal peptide sequence was supplied by Takeda Chemical Industries (Osaka, Japan). Ham's F12 nutrient medium, Opti-MEM, XO, and penicillin-streptomycin mixture were purchased from Flow Laboratories (MD, U.S.A.), Life Technologies Inc., (NY, U.S.A.), Boehringer Mannheim GmbH (Mannheim, Germany), and Bio Whittaker (ND, U.S.A.), respectively. Restriction enzymes (HindIII and XbaI), and primers 1, 2, 3, and 4 for PCR were purchased from Takara Shuzo (Kyoto, Japan). Trypsin solution, X, genistein disulfate (G418), and trypan blue were purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals were reagent grade.

Construction of a Vector Encoding the IL-2 Signal Peptide DNA Sequence-SOD cDNA (pRc/CMV-ILSOD) We constructed a secretable hSOD expression plasmid (pRc/CMV-ILSOD) using the pRc/CMV plasmid (Invitrogen Corporation, CA, U.S.A.) containing the cytomegalovirus (CMV) promoter, the neomycin resistance gene (neo'), the β-lactamase gene and a multicloning site (Fig. 1). ILSOD cDNA was constructed by the PCR-based gene expression. The schematic strategy for the PCR-based gene expression of ILSOD cDNA is shown in Fig. 2A. In first PCR, we used 5' terminal sense sequence of a signal peptide with a HindIII site (primer 1) and 3' terminal antisense sequence of signal peptide (primer 2) for primers and the cDNA encoding IL-2 signal peptide sequence (fragment A) for template. Fragment C was produced. Second PCR was performed with 5' terminal sense of hSOD (primer 3) and 3' terminal antisense sequence of hSOD with site for XbaI (primer 4) for primers and the DNA encoding hSOD cDNA (fragment B) for template, and produced fragment D. Final PCR was performed with fragments C and D, which share overlapping sequences at their 3' and 5' ends, respectively, and with primers 1 and 4. PCR produced the completed assembly shown as product E. This product was digested with HindIII and XbaI. ILSOD cDNA was inserted into the pRc/CMV vector as described. ILSOD cDNA was verified by DNA sequencing (Applied Biosystems model 373A sequencer using oligonucleotide primers and Taq cycle method, Applied Biosystems, CA, U.S.A.). The ILSOD cDNA was judged the correct nucleotide sequences of SOD cDNA, IL-2 signal sequence and 11 amino acids

Fig. 1. Structure of the Expression Vector
ILSOD cDNA

1. Signal peptide sequence

2. SOD sequence

Primer 1: Signal sense (HindIII) : 32-mer

Primer 2: Signal antisense : 36-mer

Primer 3: SOD sense : 36-mer

Primer 4: SOD antisense (Xbal) : 32-mer

Fig. 2. Strategy for Constructing the ILSOD cDNA Gene

Primer 1, 5' terminal sense sequence of signal peptide with an HindIII site; primer 2, 3' terminal antisense sequence of signal peptide; primer 3, 5' terminal sense sequence of hSOD; primer 4, 3' terminal antisense sequence of hSOD with an XbaI site.

sequence.

Preparation of Stable Transfectant  Rat lung epithelial like cells (L2 cell, ATCC CCL 149, American Type Culture Collection, MD, U.S.A.) were transfected with pRC/CMV-ILSOD by means of lipofection (Lipofectin™ reagent, Life Technologies Inc., NY, U.S.A.). Briefly, 2 x 10^5 L2 cells were seeded in 60mm tissue culture dishes and incubated at 37°C in 5% CO₂ in Ham’s F12 nutrient medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. After 24 h, this medium was replaced with 10 μg of pRC/CMV-ILSOD and 10 μg of Lipofectin in 2 ml of Opti-MEM medium, then the cells were incubated at 37°C in 5% CO₂ for 5 h. The medium for transfection was replaced with Ham’s F12 nutrient medium containing 10% FBS and the antibiotics. After 48 h, resistant clones were selected in medium containing G418 (final conc. 400 μg/ml) for 3 weeks. The interclones difference of secratable SOD activities in these G418 resistant clones was less than 1.3 times. The single clone of highest secratable SOD activity was used in the present studies.

Western Blots  The expression of SOD protein was detected by Western blotting with a modification. Five hundred microliters of medium from host or ILSOD-L2 cells was ultrafiltrated using a Microcon® (Amicon, Inc., MA, U.S.A.). The samples were lyophilized, then suspended in 10 μl of distilled water, and was applied to a 15% sodium dodecyl sulfate (SDS)–polyacrylamide gel. After electrophoresis, the proteins were electrotransferred to an Immobilon PVDF membrane (Millipore Corp., MA, U.S.A.), and incubated with anti-human Cu, Zn-SOD whole sheep serum (The Binding Site, Birmingham, UK) at 4°C overnight. Blots were visualized using the avidin–biotin complex method with biotin labeled affinity purified rabbit anti-sheep IgG (EY Laboratories, CA, U.S.A.), streptavidin labeled horseradish peroxidase and diaminobenzidine (both from Amersham International plc, Buckinghamshire, UK).
Measurement of SOD Activity, Cell Numbers, Cellular Protein and Lipid Peroxidation Host and ILSOD-L2 cells (pRc/CMV-ILSOD plasmid transfected; 2 x 10^5 cells each) were seeded in 60 mm tissue culture dishes (Biocat®, Becton Dickinson Labware, MA, U.S.A.) coated with type I rat tail collagen and incubated at 37 °C in 5% CO2 in Ham’s F12 nutrient medium containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin for 18h. To estimate the secretable SOD activity in the medium of host and ILSOD-L2 cells, Ham’s F12 nutrient medium was replaced with serum free medium (EX-cell 300™, JRH Biosciences, Tokyo, Japan). After a 3 d incubation, the SOD activity in the media from the host and ILSOD-L2 cells was measured as described by Oyanagui et al.195 using recombinant human Cu, Zn-SOD as the standard. The number of cells was estimated by trypan blue exclusion.

To estimate the intracellular SOD activity of host and ILSOD-L2 cells, a suspension of cultured cells in distilled water was frozen at -80 °C for over 24h, thawed at room temperature, then suspensions were centrifuged at 14000 rpm for 10 min. Cellular proteins, intracellular SOD activities and the level of lipid peroxidations were measured in the supernatant.

Cellular protein levels were determined using a Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, CA, U.S.A.). Lipid peroxidation was estimated using an assay kit from Wako Pure Chemicals (Osaka, Japan), that is based upon the reaction of malondialdehyde–thiobarbituric acid.290 Lipid peroxidation was expressed as malondialdehyde (MDA) equivalents per cell.

Host and ILSOD-L2 cells (2 x 10^5 cells each) were seeded in 60 mm Biocat® dishes, then incubated at 37 °C in 5% CO2 in Ham’s F12 nutrient medium containing 10% FBS, and antibiotics for 18h. To avoid XO inhibitors in FBS, Ham’s F12 nutrient medium was replaced with serum free medium (EX-cell 300™) containing 0.4 mm X. A final concentration 5 mU/ml of XO was added in the dishes.12,211 After a 1 d incubation with X/XO, the number of surviving cells was estimated by trypan blue exclusion.

Preparation of Conditioned Medium Incubated with ILSOD and Host Cells Host and ILSOD-L2 cells (3 x 10^5 cells each) were seeded in 100 mm Biocat® dishes and incubated at 37 °C in 5% CO2 in 10 ml of Ham’s F12 nutrient medium containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin for 3d. This medium was replaced with serum free medium (EX-cell 300™) containing 0.4 mm X, and then the cells were incubated for 24 and 48h. Thereafter, the cell density in the conditioned medium was adjusted to 1 x 10^4, 5 x 10^4, and 1 x 10^5 cells/ml with EX-cell 300™. These cells were counted by means of trypan blue exclusion. L2 cells (2 x 10^5 cells) were seeded in 35 mm Biocat® dishes and incubated at 37 °C in 5% CO2 in 2 ml of Ham’s F12 nutrient medium containing 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin for 24h. This medium was replaced with 2 ml of the conditioned medium of host or ILSOD-L2 cells with a final XO concentration of 5 mU/ml and the cells were incubated for a further 24h. The effect of the conditioned medium was estimated as the number of surviving cells and the amount of peroxide product.

Data Analysis Statistical analysis was performed by Student’s t-test and ANOVA. A level of p<0.05 was considered statistically significant.

RESULTS

Western Blots of Secretable SOD We constructed a secretable hSOD eukaryotic expression vector (pRc/CMV-ILSOD vector). Rat lung epithelial like cells (L2 cell) were transfected with pRc/CMV-ILSOD by means of lipofection. Western blots showed that the medium from the host cells contained one protein, whereas that from ILSOD-L2 cells had two (lanes 1 and 2 in Fig. 3). The blot in lane 3 in Fig. 3 is control recombinant hSOD. The anti-human Cu, Zn-SOD whole sheep serum was applied to these Western blots. This serum indicated the cross reactivities to SOD of human, rat, mouse and other animals. The blots with a lower molecular weight from host and ILSOD-L2 cells probably cross-reacted with endogenous rat SOD. The high molecular weight component from the ILSOD-L2 cells was not the same as that in control recombinant hSOD, because this secretable hSOD was attached to 11 amino acids of the N-terminal sequence of IL-2. Therefore, the immunoreactive SOD secreted from ILSOD-L2 cells may be hSOD fused with IL-2 N-terminal peptides.

Intracellular and Extracellular SOD Activities of ILSOD-L2 Cells The intracellular SOD activity of ILSOD-L2 cells was about 1.5 times higher than that of the host cells (Fig. 4A). Furthermore, the extracellular SOD activity of ILSOD-L2 cells was 3 times as high as that of host cells (Fig. 4B). One milligram of protein was equal to about 10^7 cells. Therefore, the amount of SOD

![Fig. 3. Western Blot of Secretable SOD Protein in Cell Cultured Medium](image-url)

Lane 1, cell cultured medium of host cells; lane 2, cell cultured medium of ILSOD-L2 cells; lane 3, recombinant hSOD (1 ng).

![Fig. 4. Intracellular and Extracellular SOD Activities Produced by ILSOD Gene Transfection](image-url)

A) Intracellular SOD activities. B) SOD activities in incubated medium. Each bar represents the mean ± S.E. (n = 3 – 10). Statistical analysis was performed by the t-test. ***p<0.005 compared with host cells.
Fig. 5. Protective Effect of IL5OD Gene Transfection against X/O-Induced Cytotoxicity

A) % surviving cells numbers. X 0.4 mm and XO 5 mU/ml. Each bar represents the mean ± S.E. (n = 6). ■ host; □ IL5OD-L2 cells. Control was the absence of XO. B) Concentration of MDA generated in the cells. Each bar represents mean ± S.E. (n = 3–6). □ control; □ X 0.4 mm and XO 5 mU/ml. Statistical analysis was performed by t-test for A and ANOVA for B. ** p < 0.01.

Fig. 6. Protective Effects of Conditioned Medium against X/O-Induced Cytotoxicity in L2 Cells

A) Effect of the number of incubated cells (10^4 and 10^5 cells/ml) upon the conditioned medium. Both host and IL5OD-L2 cells were incubated for a further 24 h. B) Effect of incubation period (24 and 48 h) on the conditioned medium. The cell density in the conditioned medium from host and IL5OD-L2 cells was adjusted to 5 x 10^4/ml. Each bar represents the mean ± S.E. (n = 3–12). X 0.4 mm and XO 5 mU/ml. Control was the absence of XO. ■ conditioned medium without prior incubation; □ conditioned medium from host cells; □ conditioned medium from IL5OD-L2 cells. Statistical analysis was performed by ANOVA. ** p < 0.01.

secreted from IL5OD-L2 cells per day was about twice the intracellular SOD content. These results indicated that IL5OD-L2 cells synthesized SOD, then secreted it into the extracellular space.

Protective Effect of IL5OD Gene Transfection against X/O-Induced Cytotoxicity and the Production of Lipid Peroxidates In this study, we generated superoxide anions in the extracellular space using X/O. We investigated the effect of intracellular and extracellular SOD activity on the extracellular superoxide anion-induced cytotoxicity generated by X/O, by transfecting rat lung cells with the SOD gene. Host and IL5OD-L2 cells were killed by X/O, although the sensitivity of IL5OD-L2 cells to X/O induced cytotoxicity decreased significantly compared with that of host cells (Fig. 5A). These findings indicated that IL5OD gene transfection protected against oxidant stress induced by X/O.

The production of lipid peroxides (MDA) in host cells in the presence of X/O increased to about double that of the control (absence of X/O) level (Fig. 5B). However, that of IL5OD-L2 cells did not change in the presence of X/O. Therefore, IL5OD-L2 cells were resistant to lipid peroxidation induced by X/O.

Protective Effects of Conditioned Medium on X/O-Induced Cytotoxicity We investigated the effect of extracellular SOD secreted from IL5OD-L2 cells against cytotoxicity induced by extracellular superoxide anions in other cells. Host and IL5OD-L2 cells were incubated for 24 and 48 h. L2 cells were then incubated with the conditioned medium from host or IL5OD-L2 cells in the presence of X/O. The 24 h conditioned medium from 10^4 host and IL5OD-L2 cells had no significant effect against X/O induced cytotoxicity (Fig. 6A). However, that from 10^5 IL5OD-L2 cells protected L2 cells against X/O induced cytotoxicity.

Figure 6B shows the effect of the pre-incubation period on cell survival. The 24 h conditioned medium from both host and IL5OD-L2 cells had no effect. However, the 48 h conditioned medium from IL5OD-L2 cells protected L2 cells against X/O induced cytotoxicity. These findings showed that the effect of the conditioned medium against superoxide anion-induced cytotoxicity was dependent upon the number of IL5OD-L2 cells and the incubation period. Thus, extracellular SOD secreted from IL5OD-L2 cells protected other cells against superoxide anion-induced cytotoxicity.

Effects of Conditioned Medium on X/O-Induced Production of Lipid Peroxidates When L2 cells were in-
cubated in the conditioned medium from ILSOD-L2 cells, less lipid peroxidates (MDA) were produced than in that from host cells (Fig. 7A). These findings indicated that secretable SOD in the extracellular space protected against superoxide anion-induced cytotoxicity. Furthermore, the relationship between superoxide anion-induced cytotoxicity in both cell types and the amount of lipid peroxidates was negative (Fig. 7B).

**DISCUSSION**

The construction of a secretable protein is a novel approach to protein delivery. This study demonstrated that SOD, normally located in the cytosol, can be engineered for secretion into the extracellular space. Many eukaryotic proteins destined for secretion are synthesized with an extra hydrophobic amino acid sequence near the amino terminal of the protein. We constructed a secretable SOD eukaryotic expression vector which expresses hSOD cDNA by fusing it to the signal peptide DNA sequence of the human IL-2 gene and 11 amino acids of N-terminal end of IL-2. This secretable SOD expression vector was transfected into cultured rat lung cells. Sasada et al. constructed several expression vectors for secretion of human EGF or IgG in mammalian cells. These vectors included human EGF or IgG cDNA, a signal sequence of IL-2, and 3 kinds of connecting peptides (1, 4 or 11 amino acids of N-terminal end of IL-2). The transformed cells with the vectors which included 11 amino acids of N-terminal end of IL-2 was secreted largest amount of EGF or IgG. These results indicated that not only the signal peptide but also a specific amino acid sequence and/or a conformation of polypeptides suitable for the signal peptide may be necessary for the efficient secretion of a foreign protein since these vector had the same IL-2 signal sequence. Hughes et al. constructed a fusion gene of β-lactamase signal sequence cDNA and human adenosine deaminase (ADA). This fusion gene did not include the connecting peptides. The fibroblasts transfected with this fusion gene demonstrated secreted ADA activity. The molecular weight of the secretable ADA was larger than non-secretable ADA, because failure of the signal sequence to be cleaved may have caused the increase in the size of ADA. Cleavage site of signal sequence by signal peptidase was influenced by length of the hydrophobic region and secondary structure formation downstream of the cleavage site. Heijne reported that a signal sequence cleavage site may be identified on the presence of a small neutral amino acid at position-1 and the absence of a large polar, aromatic, or charged residue at position-3. Therefore, it is very difficult to confirm the exact cleavage site of a signal sequence. The molecular weight of the SOD secreted from ILSOD-L2 cells was higher than that of recombinant hSOD (Fig. 3). The secretable SOD may be a fusion protein of hSOD and the 11 N-terminal amino acid residues of IL-2.

The intracellular SOD activity of ILSOD-L2 cells was about 1.5 times higher than that of host cells (Fig. 4A). Furthermore, secretable SOD activity was demonstrated in the medium from ILSOD-L2 cells, at a level that was 3 times higher than that of the host cells. Therefore, the ILSOD pReCMV vector was expressed and ILSOD-L2 cells secreted the 11 N-terminal amino acid residues–SOD fusion protein with SOD activity.

X/XO stimulates the extracellular superoxide anion concentration. We designed two in vitro models. First, we investigated the direct cytotoxic effects of superoxide anions upon cells. Host and ILSOD-L2 cells were killed by X/XO, although the sensitivity of the ILSOD-L2 cells to X/XO induced cytotoxicity decreased significantly compared with that of host cells (Fig. 5A). The production of lipid peroxidates by the host in the presence of X/XO increased to about double that of control (absence of X/XO) levels (Fig. 5B). However, in the presence of X/XO, the production of lipid peroxidates by the ILSOD-L2 cells did not change relative to control levels. Both intracellular and extracellular SOD activities increased significantly compared with those of host cells. The increase of intracellular SOD activity indicates resistance to the direct cytotoxicity of superoxide anions. Therefore, this study indicated that the increase of intracellular and/or extracellular SOD activity is mainly associated with the
resistance of ILSOD-L2 cells to superoxide anion induced cytotoxicity.

We investigated the effect of secreted extracellular SOD by ILSOD-L2 cells on the extracellular superoxide anion induced cytotoxicity and lipid peroxidation of other cells (Fig. 8). The conditioned medium from SOD-L2 cells protected against X/O induced cytotoxicity to L2 cell (Fig. 6). Therefore, when L2 cells were incubated in the conditioned medium from ILSOD-L2 cells, less lipid peroxides of L2 cells were produced than in that of host cells (Fig. 7A). The protection conferred by the conditioned medium was dependent upon the numbers of ILSOD-L2 cells and the incubation period (Figs. 6 and 7). Therefore, the SOD secreted from transformed cells can be delivered to damaged cells, and the cytotoxicity induced by superoxide anions in normal cells can be prevented by secreted SOD.

A model of cultured cells transfected with plasmid DNA is important for understanding expression. This model can be used to support animal studies and clinical trials and should be extended to animal models in vivo, to determine its clinical efficacy.

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