

Original Article

## Stanniocalcin-1 protects bovine intestinal epithelial cells from oxidative stress-induced damage

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Chronic enteritis can produce an excess of reactive oxygen species resulting in cellular damage. Stanniocalcin-1(STC-1) reportedly possesses anti-oxidative activity, the aim of this study was to define more clearly the direct contribution of STC-1 to anti-oxidative stress in cattle. In this study, primary intestinal epithelial cells (IECs) were exposed to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) for different time intervals to mimic chronic enteritis-induced cellular damage. Prior to treatment with 200 μM H<sub>2</sub>O<sub>2</sub>, the cells were transfected with a recombinant plasmid for 48 h to over-express STC-1. Acridine orange/ethidium bromide (AO/EB) double staining and trypan blue exclusion assays were then performed to measure cell viability and apoptosis of the cells, respectively. The expression of STC-1 and apoptosis-related proteins in the cells was monitored by real-time PCR and Western blotting. The results indicated that both STC-1 mRNA and protein expression levels positively correlated with the duration of H<sub>2</sub>O<sub>2</sub> treatment. H<sub>2</sub>O<sub>2</sub> damaged the bovine IECs in a time-dependent manner, and this effect was attenuated by STC-1 over-expression. Furthermore, over-expression of STC-1 up-regulated Bcl-2 protein expression and slightly down-regulated caspase-3 production in the damaged cells. Findings from this study suggested that STC-1 plays a protective role in intestinal cells through an antioxidant mechanism.

**Keywords:** Bcl-2, chronic enteritis, oxidative damage, stanniocalcin-1

### Introduction

Young calves are highly susceptible to infection by intestinal organisms. These pathogens are important causes of chronic enteritis - a disease associated with dyspepsia and diarrhea. The mechanism underlying this type of chronic inflammation remains largely unknown. Recent reports have linked the over-production of reactive oxygen species (ROS) to the development of chronic enteritis due to oxidative cellular damage [4,18].

It is often very difficult to diagnose chronic enteritis in neonatal calves by conventional methods. Current diagnostic techniques are limited to monitoring clinical symptoms and characteristic pathologic changes. Comparison of gene expression patterns for some characteristic cytokines like interleukin [16] in healthy animals and ones with chronic enteritis using molecular biological techniques has also been a reliable strategy. Recent studies have shown that STC-1, a hormone originally identified in bony fish [25] and then in mammals [5], suppresses superoxide generation [12], suggesting the involvement of STC-1 in anti-inflammation and anti-apoptosis activities. In some models of inflammation, increased expression of STC-1 relative to wild-type counterparts has been observed [22]. This finding may provide a new method for diagnosing chronic enteritis in calves by evaluating the alterations of STC-1 expression. STC-1 has also been utilized as a potential blood marker for predicting tumor aggressiveness [1], implying that the concentration of this molecule in serum has important

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clinical significance. Additionally, the fact that STC-1 can suppress superoxide generation [12] might offer a new approach to limiting chronic enteritis-induced damage in neonates.

STC-1 is a secreted glycoprotein that functions in fish as a regulator of  $\text{Ca}^{2+}/\text{PO}_4^{3-}$  homeostasis [24]. In mammals, STC-1 is normally undetectable in the blood but ubiquitously expressed in several tissues, implying that this protein maybe act as a pleiotropic factor in a paracrine/autocrine manner [6]. However, STC-1 was recently identified in the blood of young calves starting at birth [23]. Therefore, the goal of our investigation was to assess the potential relationship between STC-1 and chronic enteritis.

This study used the primary intestinal epithelial cells (IECs) obtained from neonates was due to the *in vitro* proliferative potential of them was higher than that of commercially available IEC cell lines those are thought to be inadequate models of the intact epithelium because long-term growth *in vitro* requires morphological and biochemical changes.

## Materials and Methods

The experimental protocol was approved by the official Committee on the Ethics of Animal Experiments of Huazhong Agricultural University (China).

### Cell culture reagents

The growth medium of IEC was a high-glucose formulation of Dulbecco's modified Eagle medium (DMEM; HyClone Laboratories, USA) supplemented with 5% fetal bovine serum (Hyclone Laboratories), 100 U/mL penicillin (Sigma, USA), 100  $\mu\text{g}/\text{mL}$  streptomycin (Sigma), 5  $\mu\text{g}/\text{mL}$  insulin (Sigma), 10 ng/mL epidermal growth factor (Sigma), 4 mM glutamine (Gibco BRL, USA), and 100  $\mu\text{g}/\text{mL}$  porcine mucosal heparin (Sigma). Duodenum tissues and cultured cells were rinsed with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ -free phosphate buffered saline (PBS, pH 7.2 ~ 7.4) containing 8.00 g of NaCl, 0.20 g of KCl, 3.93 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ , and 0.20 g of  $\text{KH}_2\text{PO}_4$  in 1 L of double distilled  $\text{H}_2\text{O}$ .

### Primary IEC isolation, separation, and characterization

Male 1-day-old Chinese Holstein calves (Kangli dairy farm, China) were anesthetized and euthanized with an electric shock apparatus (Qingdao ITO Food Machinery Manufacture, China) prior to nursing. Approximately 10 cm of duodenum was aseptically removed and eliminated the mesentery. The duodenum was then placed into a freezer bottle (Schott duran, Germany) containing chilled serum-free DMEM supplemented with penicillin (1,000 IU/mL) and streptomycin (1,000  $\mu\text{g}/\text{mL}$ ).

The IEC used in this study was cultured with an approach based on a previous report [10].

Viable epithelial cells were separated from contaminating fibroblasts in the primary cultures as previously described by Moyer [20]. Growth medium containing 25 U/mL collagenase I (Sigma) was used to inhibit the growth of the remaining fibroblasts as previously described [8]. After being subcultured for 3 days, the collagenase-supplemented medium was replaced with normal growth medium. The nuclei and cytoplasm of the cells were stained with H&E double staining kit (Boster Biological Technology, China) following the manufacturer's protocol. An inverted microscope (Olympus IX71; Olympus Corporation, Japan) and scanning electron microscopy (Hitachi SU8010; Hitachi High-Technologies Corporation, Japan) were used to investigated the morphology of cells in the primary cultures as previously described [14].

Immunocytochemical staining was performed to distinguish cells of epithelial origin from contaminating fibroblasts growing in the primary cultures. The purified primary cultured cells, Chinese V79 hamster lung fibroblasts (as a negative control; ATCC, USA), and rat IEC-6 intestinal crypt epithelial cells (as a positive control; ATCC) were seeded on sterile collagen-coated coverslips (Sail brand, China). The cells were incubated at 37°C for 1 h with the mouse monoclonal antibodies directed against mouse vimentin (a type III intermediate filament protein specifically expressed in fibroblast), pan-cytokeratin (PCK, intermediary type II filaments specifically expressed in epithelia), and cytokeratin 18 (CK18, intermediary type I filaments specifically expressed in epithelia) [1 : 400 in PBST (0.5% Tween 20 in 0.02 M PBS); all three antibodies were purchased from Boster Biological Technology]. Primary antibody binding was visualized with biotinylated goat anti-mouse IgG (Abcam, USA) 1 : 8,000 dilution for 1 h at room temperature (RT) and a Vectastain ABC peroxidase detection system (Vector Laboratories, USA). An inverted microscope was used to examine the stained cells.

### Construction of the recombinant pcDNA3.1/STC-1 plasmid

Total mRNA was obtained from bovine kidney using TRizol reagent (Invitrogen, USA) according to the manufacturer's instructions. A reverse transcription kit (Takara Bio, Japan) was used to synthesize cDNA. The full-length coding sequence of bovine STC-1 (Genebank Accession No. NM\_176669.3) was amplified by PCR using the following primers: 5'-ATCAAGCTTATGCT CCAAACCTCAGCAG-3' (sense) and 5'- ATTCTAGA CTAGGACTCTCTGGGAG-3' (anti-sense). Reactions consisted of 1× PCR buffer (containing 10 mM Tris-HCl, 50 mM KCl and 0.1% TritonX-100), 200  $\mu\text{M}$  for each dNTP, 2  $\mu\text{g}$  cDNA template, 1.5 mM  $\text{MgCl}_2$ , 1.5 U Taq Plus DNA

Polymerase (all of the PCR reagents were purchased from Takara Bio), and 0.2  $\mu\text{M}$  forward and reverse primers (Sangon Biotech, China). After denaturation at 94°C for 5 min, the reaction proceeded for 30 cycles of 94°C for 15 sec, 60°C for 15 sec and 72°C for 25 sec. And finally extended for 5 min at 72°C. The amplicon was cloned into a pcDNA3.1<sup>+</sup> plasmid (Invitrogen) at the *HindIII* and *XbaI* (Takara Bio) sites. The recombinant plasmid was named pcDNA/STC-1. The pcDNA/STC-1 plasmid was then transformed into DH5 $\alpha$  cells (Invitrogen) and the cells were cultured in Lysogeny broth medium at 37°C overnight.

A commercial kit (Omega Bio-Tek, USA) was used to extract the endotoxin-free plasmid from overnight cultures of transformed DH5 $\alpha$  cells. The primary IECs were transfected with pcDNA/STC-1 (4.0  $\mu\text{g}/\text{well}$ ), an equivalent amount of pcDNA3.1<sup>+</sup> vector, or an equivalent volume of PBS in lieu of plasmid (as control) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Total mRNA and protein were harvested from the cells at 0, 24, 48, and 72 h post-transfection.

### Induction of cell damage

To mimic damage associated with chronic enteritis, the IECs were incubated at 37°C and treated with or without 200  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> in growth medium for different periods of time (4, 8, 12, 16, and 24 h).

To determine the effect of STC-1 on H<sub>2</sub>O<sub>2</sub>-induced damage, the IECs were divided into six groups. Cells in the first group were incubated at 37°C in growth medium without H<sub>2</sub>O<sub>2</sub> for 56 h (as control group). IECs in the second group were cultured for 48 h in growth medium and then treated with 200  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> for 8 h. Cells in the third and fourth groups were transfected with pcDNA/STC-1 or pcDNA3.1<sup>+</sup>, respectively, and then cultured in growth medium for 56 h. Finally, cells in the fifth and sixth groups were transfected with pcDNA/STC-1 or pcDNA3.1<sup>+</sup>, respectively; cultured in growth medium for 48 h, and then treated with 200  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> for 8 h.

Total mRNA and proteins were harvested from a portion of the treated cells, and the other treated cells were detached with 0.04% (w/v) trypsin (Sigma) for a cell viability test.

### Cell viability test

Viability of the IECs treated with or without H<sub>2</sub>O<sub>2</sub> was evaluated by double staining with AO/EB (Sigma). After removing the H<sub>2</sub>O<sub>2</sub>, the cells were washed with PBS and trypsinized. A mixture containing 100  $\mu\text{L}$  of the cell suspension containing about 10<sup>5</sup> cells/mL in PBS and 10  $\mu\text{L}$  of the AO/EB working solution (50  $\mu\text{g}/\text{mL}$  of AO and 50  $\mu\text{g}/\text{mL}$  of EB in PBS) was then prepared. The cells were immediately analyzed with a fluorescence microscope (Olympus IX71; Olympus Corporation). Cell death rate was monitored by trypan blue exclusion with a hemocytometer

(Nexcelom Bioscience, USA) as previously described [19].

### Quantitative real-time PCR

Total RNA extraction and cDNA preparation were performed for each sample in the manner detailed above. Amplification and quantification were performed by a StepOne Real-Time PCR System (Applied Biosystems, USA) with SYBR Green I. Sequences of the primers for STC-1 were 5'-AGTGATTCGCTGCCTCAACA-3' (sense) and 5'-TCTCCAGGCATGCAAAAGCT-3' (anti-sense). Sequences of the primers for GAPDH (as a control) were 5'-CCTTCATTGACCTTCACTACATGGTCTA-3' (sense) and 5'-TGGAAGATGGTGATGGCCTTCCATTG-3' (anti-sense). Reactions consisted of 1  $\times$  SYBR Green I (Takara Bio), 0.2  $\mu\text{M}$  forward and reverse primers (Sangon Biotech) for each gene, and 1/50 of total reaction volume of ROX. The thermal cycling conditions were 5 min at 94°C followed by 40 cycles of 20 sec at 94°C, 20 sec at 60°C for each gene, and 15 sec at 72°C with a final 10 min extension at 72°C.

### Western blot analysis

Cells were lysed for 10 min with ice-cold lysis kit (Beyotime Institute of Biotechnology, China) supplemented with a protease inhibitor mixture (Sigma). An 8-min centrifugation was performed at 12,000  $\times$  g to collect the supernatant whose protein concentration was measured using a BCA Protein Assay kit (Beyotime Institute of Biotechnology). A total of 30  $\mu\text{g}$  of proteins from each sample were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, USA). The membranes were then blocked with 5% (w/v) non-fat milk (CWbio, China) in PBST for 2 h at RT. Next, the blots were incubated with rabbit polyclonal antibodies against human STC (1 : 500 in PBST; Abcam), Bcl-2 (1 : 600; Sangon Biotech), Bax (1 : 600; Sangon Biotech), caspase-3 (1 : 600; ABclonal Biotechnology, China), and GAPDH (1 : 5,000; Abcam) at RT for 2 h. The membranes were subsequently washed six times with PBST for 5 min at RT and then incubated with goat anti-rabbit IgG-HRP for 2 h at RT (1 : 8,000; CWbio). The immunoreactive bands were visualized using an Enhanced chemiluminescence (ECL) kit (CWbio).

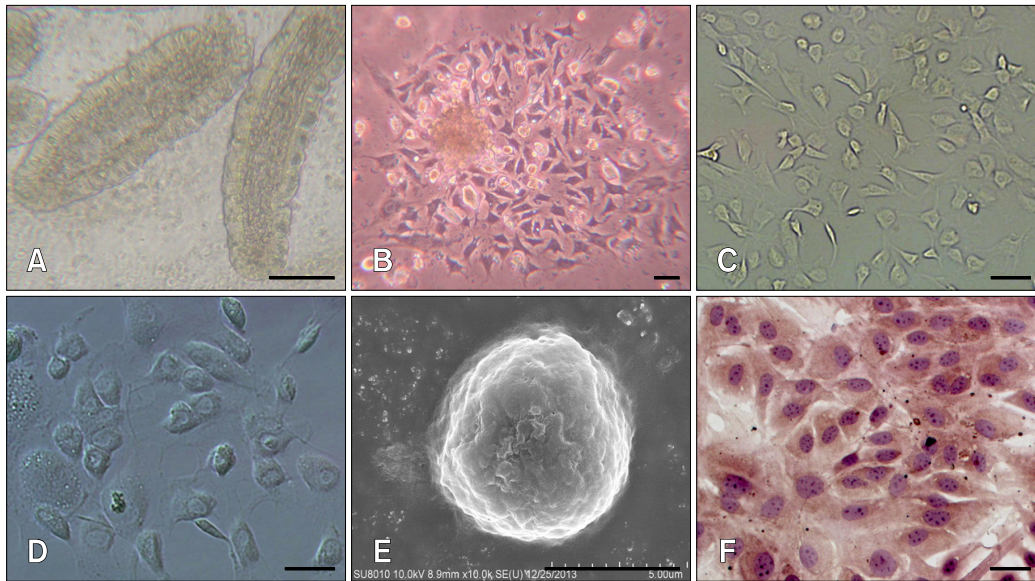
### Statistical analysis

Data were analyzed using a one-way ANOVA for each triplicate or quadruplicate set using SPSS Statistics (SPSS, USA). Values for all parameters are expressed as the mean  $\pm$  standard error of the mean (SEM). A *p* value < 0.05 was considered statistically significant.

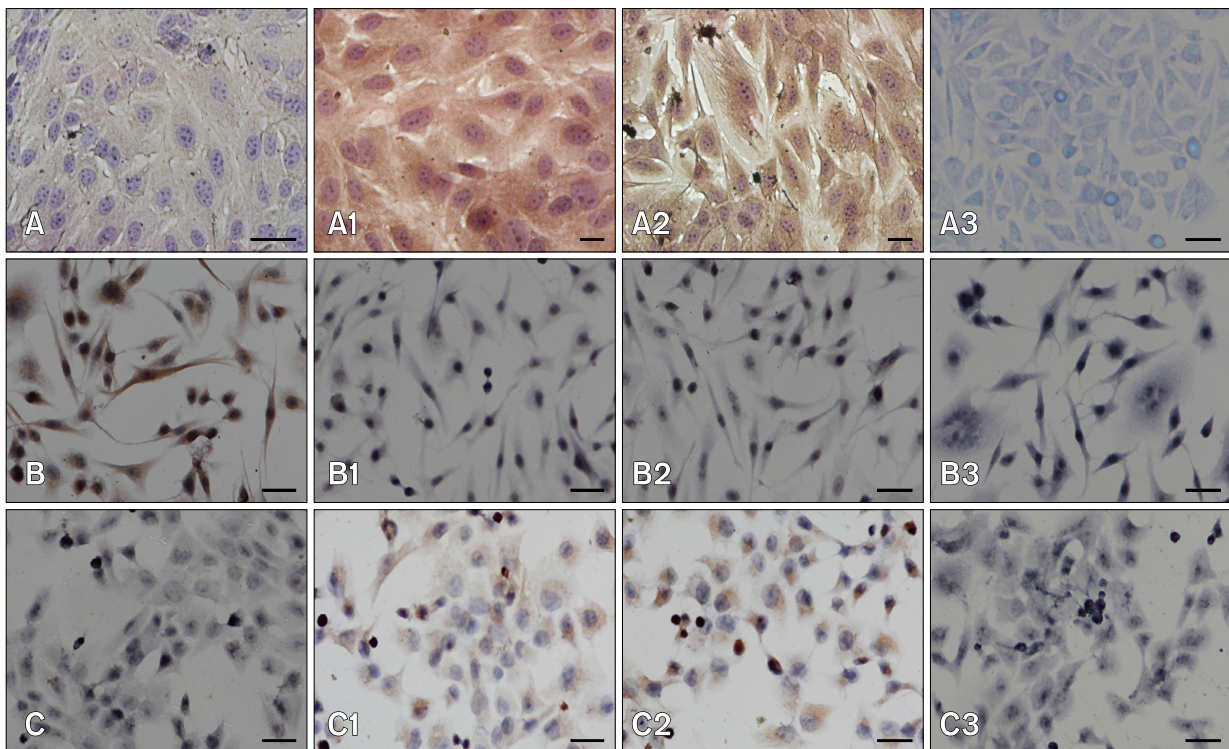
## Results

### Isolation and characterization of the IECs

The best procedure for isolating IECs was formulated



**Fig. 1.** Morphology of intestinal cells in culture. Phase contrast microscopy was performed to observed characteristics associated with the evolution of bovine IECs in primary cultures starting from adherent organoid structures (A) that gave rise to larger proliferating foci (B) which joined together to form a confluent monolayer. After purification, the subcultured epithelial cells grew dispersedly in the flask (C). The individual epithelial cell had a dome-like and rounded morphology when viewed with a differential interference contrast microscope (D) or scanning electron microscope (E), respectively. The nuclei and cytoplasm of the hematoxylin- and eosin-stained cells were blue and pink, respectively (F).  $\times 40$  (A),  $\times 200$  (B ~ D, and F), and  $\times 15,000$  (E). Scale bars = 200  $\mu\text{m}$  (A) or 50  $\mu\text{m}$  (B ~ F).



**Fig. 2.** Characterization of cells stained for immunocytochemistry. Panels show the primary cultures, V79, and IEC-6 cells, and indicate cells stained with antibodies against vimentin, PCK, CK18, or PBS (as the negative control), from left to right, respectively. Note that both the primary cultures (A1 and 2) and IEC-6 cells (C1 and 2) were stained yellow by the antibodies against PCK and CK18, but not by the anti-vimentin antibody (A and C). In contrast, the V79 cells displayed opposite patterns of immunoreactivity (B ~ B2).  $\times 400$  (A1 and 2),  $\times 200$  (A, A3, B ~ B3, and C ~ C3). Scale bars = 20  $\mu\text{m}$  (A1 and 2) or 50  $\mu\text{m}$  (A, A3, B ~ B3, and C ~ C3).



based on our preliminary experiments showing that digestion with collagenase I for approximately 2 h yields high quality individual crypts or villi (panel A in Fig. 1) from intestinal epithelium. When adhered to the flask, the purified individual villi gave rise to proliferating foci of cells (panel B in Fig. 1) that formed confluent monolayers within 3~7 days. After detachment with 0.04% trypsin, reattachment, and purification, the IECs formed a homogeneous pavement-like layer typical of epithelial sheets (panel C in Fig. 1). The cells had a dome-like morphology as seen with the differential interference contrast microscope (panel D in Fig. 1), and a globose morphology with a rough surface when viewed with the scanning electron microscope (panel E in Fig. 1). The nuclei and cytoplasm of the cells stained with H&E were blue and pink, respectively (panel F in Fig. 1).

Results of the immunocytochemical staining experiment indicated that the primary IECs and IEC-6 cells exhibited strong immunoreactivity specific for PCK and CK18 (panels A1, A2, C1, and C2 in Fig. 2) but not vimentin (panels A and C in Fig. 2). In contrast, only yellow staining corresponding to vimentin was found in the V79 cells (panel B in Fig. 2); immunoreactivity against PCK or CK18 was not observed (panels B1 and B2 in Fig. 2). These results suggest that epithelial cells were the major component of the primary cultures.

### STC-1 expressed in the transfected IECs

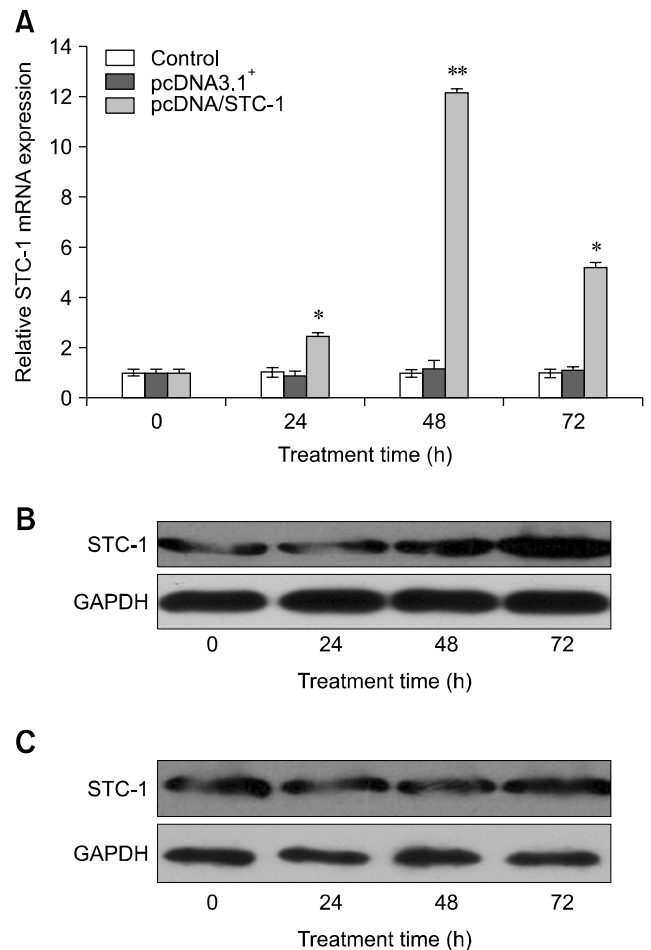
The expression of STC-1 was evaluated by real-time PCR and Western blotting. We found that transfection with pcDNA/STC-1 was effective for over-expressing STC-1. In cells containing this construct, mRNA expression levels in the cells were maximized at approximately 48 h after transfection ( $p < 0.001$ , panel A in Fig. 3) whereas protein expression was maximized at 72 h (panel B in Fig. 3). On the other hand, no significant changes in either STC-1 mRNA or protein expression were observed in cells transfected with the pcDNA3.1<sup>+</sup> vector or the PBS blank (panels A and C in Fig. 3). Ultimately, the cells were used for further study 48 h after transfection considering the expression patterns of STC-1 and cell growth.

### STC-1 over-expression and H<sub>2</sub>O<sub>2</sub>-induced cellular damage

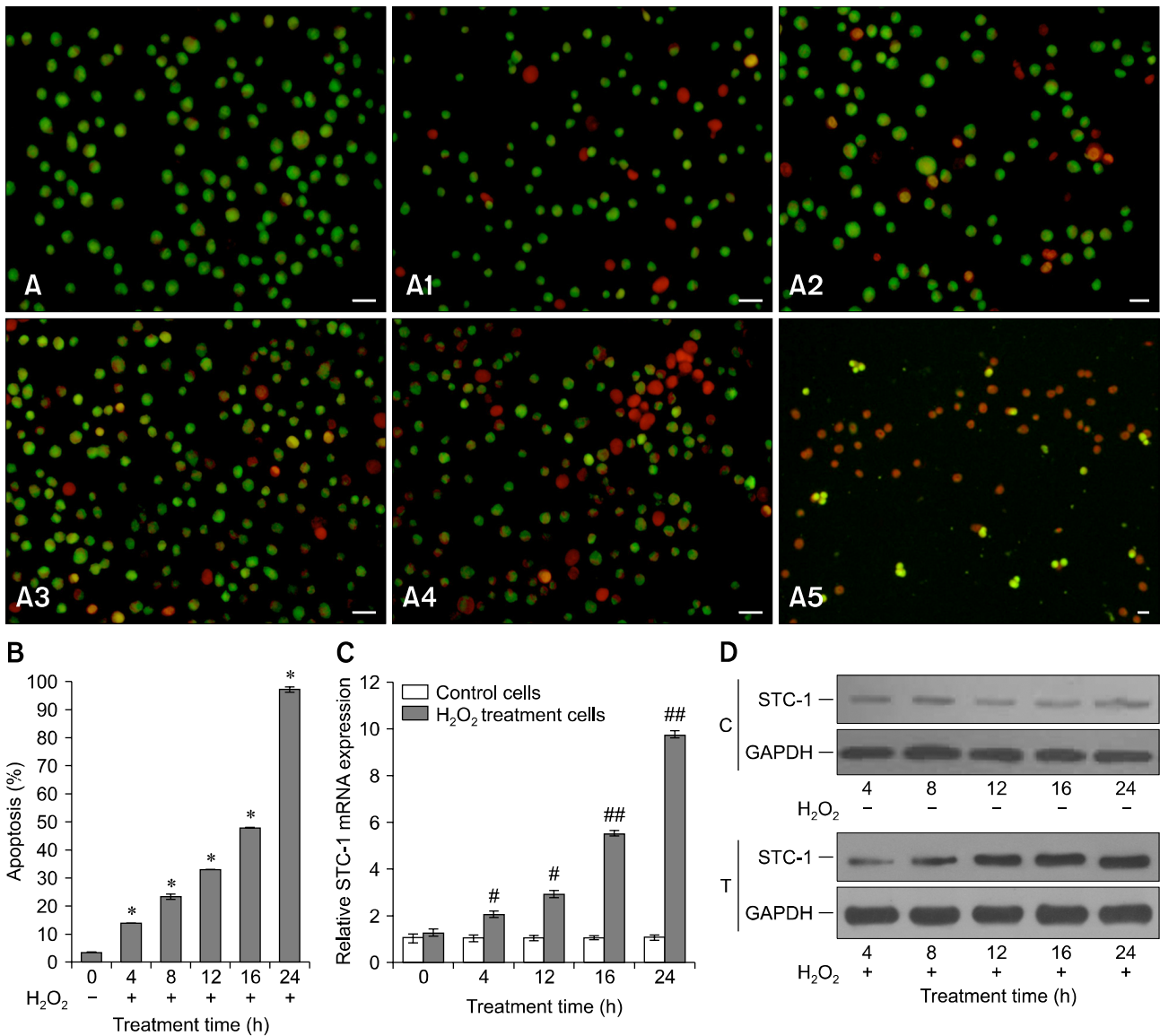
Cell viability (panels A-A5 in Fig. 4) and the cell survival rate ( $p < 0.001$ , panel B in Fig. 4) decreased in a time-dependent manner when the cells were exposed to 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> as measured by AO/EB double staining and the trypan blue exclusion assay, respectively. A time-dependent increase in STC-1 mRNA and protein expression was also observed in the cells with H<sub>2</sub>O<sub>2</sub>-induced damage ( $p < 0.01$ , panels C and D in Fig. 4), suggesting that STC-1 expression can be stimulated by oxidative stress. Decreased cell viability and survival rates

resulting from H<sub>2</sub>O<sub>2</sub> treatment were significantly improved by pre-transfection with pcDNA/STC-1 (panel A2 and the fifth column in panel B in Fig. 5,  $p < 0.01$ ). Protection against damage by H<sub>2</sub>O<sub>2</sub> was not observed in the cells pre-transfected with the pcDNA3.1<sup>+</sup> vector (panel A3 and sixth column in panel B in Fig. 5,  $p < 0.1$ ).

We also found that the expression of Bcl-2 protein was significantly down-regulated in the cells treated with H<sub>2</sub>O<sub>2</sub> alone for 8 h (panel C in Fig. 5) or H<sub>2</sub>O<sub>2</sub> for 8 h after pcDNA3.1<sup>+</sup> pre-transfection for 48 h (Lane 3 in panel C in Fig. 5) compared to the control group (Lane 1 in panel C in Fig. 5). Pre-transfection with pcDNA/STC-1 restored Bcl-2 expression (panel C in Fig. 5). Interestingly, the expression patterns of caspase 3 protein were opposite to



**Fig. 3.** Analysis of STC-1 mRNA and protein expression in the transfected IECs. The expression of STC-1 mRNA in cells transfected with the pcDNA/STC-1 construct was maximized approximately 48 h after transfection as measured by real-time PCR (gray column in A; \* $p < 0.01$  or \*\* $p < 0.001$  vs. the control cells). Protein expression was maximized at 72 h as measured by Western blotting (B). In contrast, no significant changes were observed in STC-1 mRNA or protein expression in cells transfected with the pcDNA3.1<sup>+</sup> vector (black column in A and C).



**Fig. 4.** Detection of viability, apoptosis, and STC-1 expression of transfected IECs. A time-dependent decrease in cell viability (A ~ A5, cells treated with H<sub>2</sub>O<sub>2</sub> for 0, 4, 8, 12, 16, and 24 h) and survival rate (B, \**p* < 0.001 vs. 0 h) was observed for cells treated with H<sub>2</sub>O<sub>2</sub> using AO/EB double staining and a trypan blue exclusion assay, respectively. Additionally, time-dependent increases in STC-1 mRNA (C, #*p* < 0.01 or ##*p* < 0.001 vs. the control cells) and protein expression (D, C: control cells, T: H<sub>2</sub>O<sub>2</sub>-treated cells) were found in the cells exposed to 200 μM H<sub>2</sub>O<sub>2</sub> using real-time PCR and Western blotting, respectively. ×400 (A ~ A5). Scale bars = 20 μm (A ~ A5).

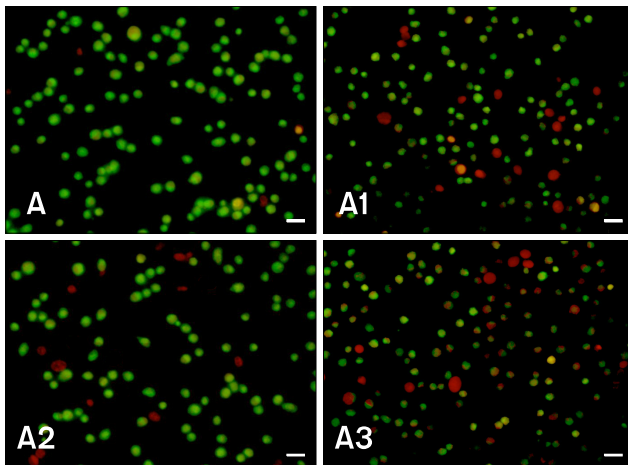
those observed for Bcl-2. Additionally, no change in the expression of Bax protein in the H<sub>2</sub>O<sub>2</sub> treatment groups was observed (panel C in Fig. 5) although expression was enhanced compared to that found in the control group (panel C in Fig. 5).

### Discussion

The major goal of our study was to elucidate the relationship between STC-1 and chronic enteritis. This investigation was restricted by a lack of adequate diseased

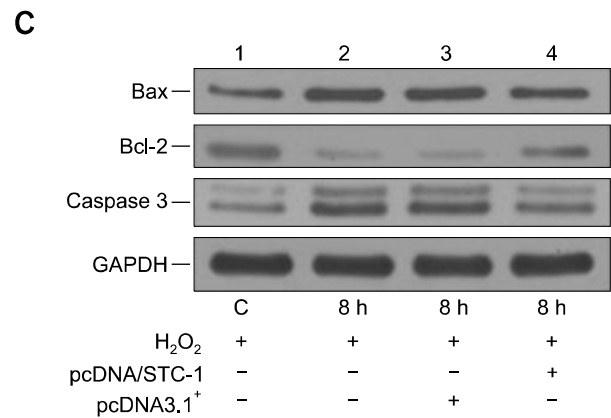
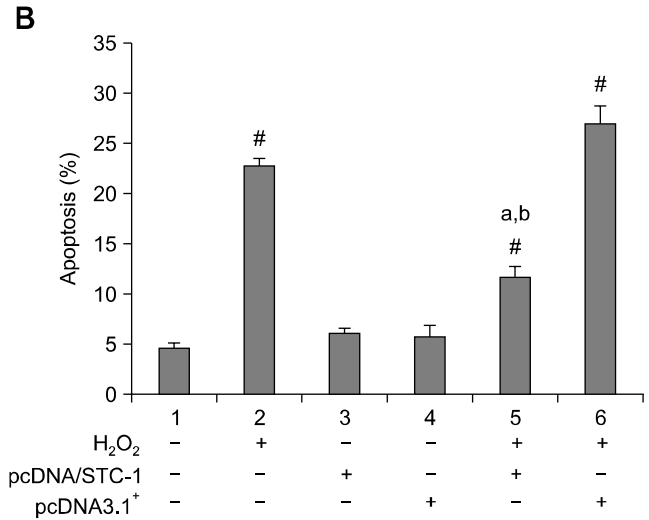
animals and difficulties associated with making *in vivo* observations. Therefore, cultured primary IECs were harvested and used for our investigation. To mimic cellular damage induced by chronic enteritis, the primary cells were treated with H<sub>2</sub>O<sub>2</sub> for 4 ~ 24 h. This strategy helped ensure the continuity of reactive oxygen species (ROS) production found during chronic inflammation, and was based on previous work by Bae *et al.* [2] and our group [28] who treated MCF-7 cells or cardiomyocytes for 24 h with 200 μM H<sub>2</sub>O<sub>2</sub> to induce apoptosis.

As reported in the literature, chronic enteritis is a common



**Fig. 5.** The effects of STC-1 over-expression on the viability, apoptosis rate, and apoptosis-related protein expression in IECs. (A~A3) Viability of cells grown normally, treated with H<sub>2</sub>O<sub>2</sub> for 8 h, or pre-transfected with pcDNA/STC-1 or pcDNA3.1<sup>+</sup> for 48 h before H<sub>2</sub>O<sub>2</sub> treatment, respectively. In cells that over-overexpressed STC-1, H<sub>2</sub>O<sub>2</sub>-induced cellular injury was significantly attenuated as measured by AO/EB double staining (A3). (B) A significant decrease of apoptosis induced by H<sub>2</sub>O<sub>2</sub> was observed in cells transfected with pcDNA/STC-1 (fifth column) as measured by a trypan blue exclusion assay (<sup>#</sup>*p* < 0.01 vs. the control; <sup>a</sup>*p* < 0.01 vs. cells treated with H<sub>2</sub>O<sub>2</sub> alone; <sup>b</sup>*p* < 0.01 vs. the pcDNA3.1<sup>+</sup>-transfected cells treated with H<sub>2</sub>O<sub>2</sub>). (C) Expression of apoptosis-related factors measured by Western blotting. Lane 1, Cells grown normally as the control; Lane 2, cells treated with H<sub>2</sub>O<sub>2</sub> alone for 8 h; Lane 3, cells treated with H<sub>2</sub>O<sub>2</sub> alone for 8 h after transfection with pcDNA3.1<sup>+</sup> for 48 h; Lane 4, cells treated with H<sub>2</sub>O<sub>2</sub> alone for 8 h after transfection with pcDNA/STC-1 for 48 h. Cells transfected with pcDNA/STC-1 showed a significant up-regulation of Bcl-2 expression and a slight down-regulation of caspase 3 expression. ×400 (A1~A4). Scale bars = 20 μm (A1~A4).

disease of several mammalian species caused by multiple factors [26]. The lack of convenient animal models and complexity of diagnosing this condition made prior studies in cattle somewhat sporadic. In previous investigations, oxidative stress was thought to be one mechanism underlying this inflammatory disorder [9] since excessive endogenous ROS produced during chronic enteritis inevitably lead to the activation of critical cellular survival pathways in IECs [27]. Theoretically, the prevention of ROS over-production and increased ROS scavenging could be an effective strategy for decreasing cell injury. STC-1 has been found to suppress H<sub>2</sub>O<sub>2</sub> production [11] and promote H<sub>2</sub>O<sub>2</sub> decomposition [3], indicating that this factor exerts an antioxidant effect in the gut during oxidative injury. Consistent with these findings, our results clearly demonstrated that STC-1 over-expression increased cell viability and attenuated the injury of bovine IECs that were exposed to H<sub>2</sub>O<sub>2</sub>. These effects may be related to the abilities of STC-1 to uncouple oxidative phosphorylation in mitochondria [11,22] and modulate the activities of some enzymes that scavenge H<sub>2</sub>O<sub>2</sub> [3]. Our



results suggest that increasing STC-1 expression might help treat chronic enteritis and prevent enterocyte apoptosis promoted by oxidative stress.

Given the complexity of chronic enteritis pathogenesis, an accurate diagnosis is very difficult to make. Monitoring the cytokines involved in inflammation could be a promising direction. For example, interleukin is a well-characterized indicator of an inflammatory response and has been widely used to diagnose various types of inflammation [13]. Previous studies demonstrated that STC-1 is normally absent from the blood [6,7]. However, recent research determined that this factor exists in the blood of calves starting at birth [23]. Therefore, measuring the level of STC-1 in blood might be a novel way to predict the development of chronic inflammatory disease. Our data indicated that the expression of STC-1 was induced by H<sub>2</sub>O<sub>2</sub> in a time-dependent manner. Thus, the level of expression was positively associated with the extent of H<sub>2</sub>O<sub>2</sub>-induced cellular damage. These results suggest that the induction of STC-1 expression might be a general adaptive and protective response to oxidative stress, and

support an earlier finding that STC-1 gene expression is stimulated by oxidative stress [21]. It is still unclear how STC-1 is up-regulated by oxidative stress and the exact mechanism underlying its protective role is still unknown. Yeung *et al.* [29] reported that the expression of STC-1 is stimulated by hypoxia inducible factor-1 (HIF-1). Law *et al.* [15] identified a putative hypoxia response element (HRE) site in the STC1 gene, suggesting that STC-1 expression is controlled by a complicated regulatory mechanism. Further study is needed to determine whether HIF-1 mediates the up-regulation of STC-1 in the presence of H<sub>2</sub>O<sub>2</sub>.

Our current investigation had several limitations. We lacked an appropriate animal model or access to animals suffering from chronic enteritis. These were major weaknesses. However, all of these problems will be addressed in future studies.

In conclusion, we determined that altered expression of apoptosis-related proteins is an effect of H<sub>2</sub>O<sub>2</sub>-induced oxidative injury. We also found that the expression of Bcl-2, a well-characterized anti-apoptotic protein, was significantly enhanced by H<sub>2</sub>O<sub>2</sub> treatment and STC-1 over-expression compared to exposure to H<sub>2</sub>O<sub>2</sub> alone. H<sub>2</sub>O<sub>2</sub> and STC-1 generation had the opposite effect on caspase 3 expression. The findings suggested that the ability of STC-1 to attenuate H<sub>2</sub>O<sub>2</sub>-induced damage is partly due to up-regulated Bcl-2 expression [17].

## Acknowledgments

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## Conflict of Interest

There is no conflict of interest.

## References

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