

The Two Isomers of HDTIC Compounds from *Astragalus Radix* Slow Down Telomere Shortening Rate via Attenuating Oxidative Stress and Increasing DNA Repair Ability in Human Fetal Lung Diploid Fibroblast Cells

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4-Hydroxy-5-hydroxymethyl-[1,3]dioxolan-2,6'-spirane-5',6',7',8'-tetrahydro-indolizine-3'-carbaldehyde (HDTIC)-1 and HDTIC-2 are two isomers extracted from *Astragalus membranaceus* (Fisch) Bunge Var. *mongholicus* (Bge) Hsiao. Our previous study had demonstrated that they could extend the lifespan of human fetal lung diploid fibroblasts (2BS). To investigate the mechanisms of the HDTIC-induced delay of replicative senescence, in this study, we assessed the effects of these two compounds on telomere shortening rate and DNA repair ability in 2BS cells. The telomere shortening rates of the cells cultured with HDTIC-1 or HDTIC-2 were 31.5 and 41.1 bp with each division, respectively, which were much less than that of the control cells (71.1 bp/PD). We also found that 2BS cells pretreated with HDTIC-1 or HDTIC-2 had a significant reduction in DNA damage after exposure to 200 μ M H₂O₂ for 5 min. Moreover, the 100 μ M H₂O₂-induced DNA damage was significantly repaired after the damaged cells were continually cultured with HDTIC for 1 h. These results suggest that HDTIC compounds slow down the telomere shortening rate of 2BS cells, which is mainly due to the biological properties of the compounds including the reduction of DNA damage and the improvement of DNA repair ability. In addition, the slow down of telomere shortening rate, the reduction of DNA damage, and the improvement of DNA repair ability induced by HDTIC may be responsible for their delay of replicative senescence.

Introduction

REPLICATIVE SENESCENCE of human fetal lung diploid fibroblasts (2BS) is caused by the exhaustion of their proliferative potential. The cellular and molecular mechanisms of replicative senescence are very complicated. It is likely to be controlled by multiple pathways, which may be independent and also overlapping (Cox, 1997). However, DNA damage and telomere shortening play a very important role in cellular senescence (Aubert and Lansdorp, 2008; Vijg, 2008). In normal culture conditions, the telomeres of human diploid fibroblasts shorten by 50–100 bp with each division (Harley *et al.*, 1990; Allsopp *et al.*, 1992). When their telomeres reach a limited length, the cells lose potential to divide and arrest in G₀ or G₁ phase. Therefore, 2BS cells should gain additional population doublings (PDs) if the rate of their telomere erosion slows down. The increased telomere erosion mainly results from the accumulation of DNA single-strand breaks induced by oxidative stress (von Zglinicki *et al.*, 1995; Bodnar *et al.*, 1998; Aubert and Lansdorp, 2008; Cattani *et al.*, 2008; Serra *et al.*, 2000). Moreover, oxidative DNA damage and DNA repair ability also

play key roles in cellular senescence via blocking the binding of transcriptional factors with the damaged DNA-binding domain and inducing cell-cycle arrest, etc. (Chen and Ames, 1994; Di Leonardo *et al.*, 1994; Chen *et al.*, 1995; Smith and Fornace, 1996; Collins *et al.*, 1997; King *et al.*, 1997).

HDTIC-1 and HDTIC-2 (see Fig. 1), two isomers of 4-hydroxy-5-hydroxymethyl-[1,3]dioxolan-2,6'-spirane-5',6',7',8'-tetrahydro-indolizine-3'-carbaldehyde (HDTIC) extracted from *Astragalus membranaceus* (Fisch) Bunge Var. *mongholicus* (Bge) Hsiao, can extend the lifespan of human fetal lung diploid fibroblasts (2BS) (Wang *et al.*, 2003). To investigate the mechanisms of HDTIC-induced delay of replicative senescence, in this study, we examined the effects of HDTIC compounds on the telomere shortening rate, the anti-oxidative potential, and the DNA repair abilities.

Materials and Methods

Cell lines

2BS cells isolated from female fetal lung fibroblast tissue have been fully characterized (Tang *et al.*, 1994). The 2BS cells

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are considered to be young at PD30 or below and to be fully senescent at PD55 or above.

HDTIC-1 and HDTIC-2

HDTIC-1 and HDTIC-2 were isolated from *Astragali Radix*. They were kindly provided as a gift by Dr. Tu Pengfei (Modern Research Center for Traditional Chinese Medicine, Peking University). The structures of the molecules were identified by mass spectrometry, ^1H -nuclear magnetic resonance and ^{13}C -nuclear magnetic resonance. HDTIC-1 and HDTIC-2 were kept at 1 mM stock solution in dimethyl sulfoxide. They were diluted to the required working concentrations using Dulbecco's modified Eagle's medium (DMEM).

Cell culture

Cells were grown in DMEM (Sigma, Santa Clara, CA), containing 53 mM NaHCO_3 and 8.85 mg/mL Hepes (Sigma), supplemented with 10% fetal calf serum and with 60 $\mu\text{g}/\text{mL}$ penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin. The cumulative PDs were calculated as $\log_2(D/D_0)$, where D and D_0 are defined as the density of cells at the time of harvesting and seeding, respectively. The last culture was defined when the subculture could not be confluent in 3 weeks.

Determination of telomere length by Southern blots

DNA extracted from 2BS cells was completely digested with the restriction enzyme *EcoR1* to produce terminal restriction fragments (TRFs) as previously described (Wang *et al.*, 2007). The digested DNA was loaded onto a 1.0% agarose gel and electrophoresized at 40 V for 8 h together with $\lambda\text{DNA}/\text{Hin III}$ digest as a size marker. DNA was dephosphorylated by soaking gels in 0.25 M HCl for 10 min, denatured in 0.2 M NaOH/0.6 M NaCl for 25 min, and transferred to a nitrocellulose membrane NYTRAN (Schleicher & Schuell, Keene, NH). DNA was prehybridized with denatured salmon sperm DNA at 50°C for 8 h and hybridized in a solution (5 \times Denhardt's, 5 \times standard saline citrate [SSC] 0.1% sodium dodecyl sulphate, 100 $\mu\text{g}/\text{mL}$ denatured salmon sperm DNA, 20 mM NaH_2PO_4 , pH 7.4) at 50°C with 5-end [^{32}P]-labeled (TTAGGG) $_4$ for 36 h. Membranes were washed in 4 \times SSC/0.1% sodium dodecyl sulphate at 56°C and underwent autoradiography with a Kodak X-ray film. The image was viewed by Image Master VDS System (Pharmacia Biosci,

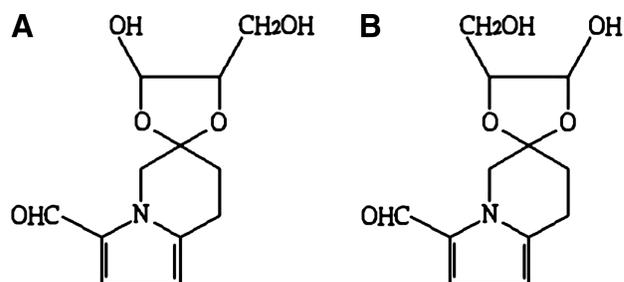


FIG. 1. Chemical structures of HDTIC-1 (A) and HDTIC-2 (B).

Uppsala, Sweden), and the length of the telomere was analyzed by Leica Image analysis system. Telomere shortening rate was calculated as $(L_{28} - L_n)/(n - 28)$, where L_n was the telomere length of 2BS cells in PD n .

HDTIC pretreatment and H_2O_2 treatment

2BS cells were cultured with DMEM containing HDTIC-1 (0.1 μM) or HDTIC-2 (1.0 μM) for 12 h at 37°C, 5% CO_2 in the dark. The cells were then washed twice with cold phosphate-buffered saline (PBS) and exposed to 200 μM H_2O_2 for 5 min at room temperature in the dark. After being washed twice with cold PBS removing the remaining H_2O_2 , the cells were digested with 0.25% trypsin and collected in PBS.

Assay for oxidative DNA strand breaks

The oxidative DNA strand breaks were measured by the single-cell gel electrophoresis (also called comet assay) technique. Single-cell gel electrophoresis was performed according to a previous publication with minor modifications (Ribeiro *et al.*, 2007). In brief, fully frosted microscopic slides were each covered with 110 μL of 0.5% normal melting agarose, and then 70 μL of 1% lower melting agarose containing 5×10^4 2BS cells was rapidly pipetted onto the first agarose layer. The slides were immersed in freshly prepared cold lysing solution (2.5 M NaCl, 100 mM Na_2 ethylenediaminetetraacetic acid, 10 mM Tris, pH 10, 1% sodium sarcosinate) with 1% Triton X-100 for 40 min at 4°C in the dark. Then slides were placed in fresh electrophoresis solution (1 mM Na_2 ethylenediaminetetraacetic acid, 300 mM NaOH,

FIG. 2. Telomere lengths in 2BS cells grown from population doubling (PD)28 in 0.1 μM HDTIC-1 or 1.0 μM HDTIC-2. Genomic DNA was digested with *EcoR1*. Telomere length was determined by Southern blots using [γ - ^{32}P]-labeled (TTAGGG) $_4$ oligonucleotide probe for terminal restriction fragments of genomic DNA. A, B, E, F, I, and L represent the telomere lengths of control cells at PD28, PD42, PD55, PD42, PD55, and PD55, respectively; C, D, and J represent the telomere lengths of cells grown from PD28 in 0.1 μM HDTIC-1 at PD42, PD55, and PD74, respectively; G, H, and K represent terminal restriction fragment lengths of the cells treated with 1.0 μM HDTIC-2 at PD42, PD55, and PD69, respectively.

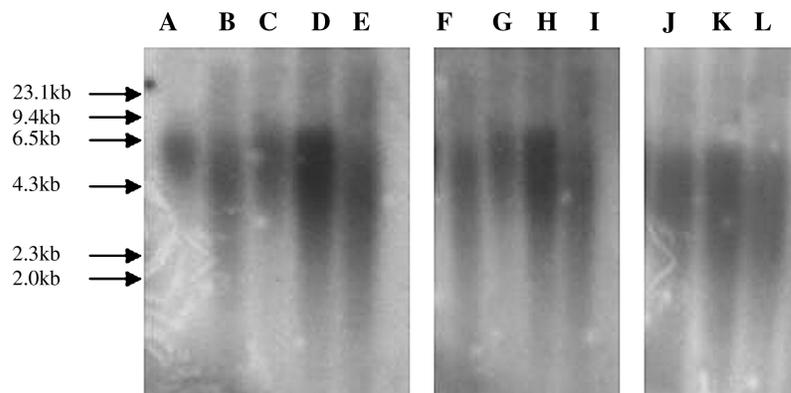


TABLE 1. EFFECTS OF HDTIC-1 AND HDTIC-2 ON TELOMERE LENGTHS (MEAN \pm STANDARD DEVIATION), $N=3$

Treatment	PD28 (kb)	PD35 (kb)	PD42 (kb)	PD50 (kb)	PD55 (kb)	Telomere shortening rate (bp/PD)
Control	8.93 \pm 0.54	8.60 \pm 0.77	8.21 \pm 0.52	7.65 \pm 0.46	7.01 \pm 0.43	71.1 \pm 4.9
0.1 μ M HDTIC-1	8.93 \pm 0.54	8.70 \pm 0.55	8.56 \pm 0.50	8.24 \pm 0.57	8.08 \pm 0.49	31.5 \pm 2.4 ^a
1.0 μ M HDTIC-2	8.93 \pm 0.54	8.65 \pm 0.63	8.37 \pm 0.46	8.05 \pm 0.58	7.82 \pm 0.41	41.1 \pm 3.5 ^a

The telomere lengths in 2BS cells grown from PD28 in HDTIC-1 (0.1 μ M) or HDTIC-2 (1.0 μ M) were analyzed. Mean terminal restriction fragment length was estimated as the center of mass and expressed in kb \pm standard deviation based on the equation: $\sum(MWi \times ODi) / \sum(ODi)$, where ODi is the densitometric output and Mwi is the length of the DNA at position i. Data are typical of three independent experiments.

Compared with control, ^a $p < 0.05$.

PD, population doubling.

pH 13.0) for 10 min to allow the unwinding of the DNA. Electrophoresis was conducted at 4°C for 25 min using 15 V (1 V/cm). Seventy-five microliters of propidium iodide (5 μ g/mL) was added to each slide and incubated overnight.

Slides were examined at 100 \times magnification and pictures were taken using a fluorescence microscope (TCS.SPZ, Leica, Mannheim, Germany). The image was evaluated by an image analysis system (Q550CW, Leica). The percentage of comet tail area (tail area/total area) and the tail length (from the center of the head to the end of the tail) were analyzed in 100 cells per slide.

DNA repair ability

2BS cells were washed twice with PBS and then exposed to 100 μ M H₂O₂ for 5 min in the dark. The cells were washed twice by PBS to remove H₂O₂ completely and continually cultured with normal DMEM (control) or HDTIC-supplemented DMEM for 1 h. The cells were harvested and analyzed with comet assay.

Statistical analysis

Statistical analyses of the telomere data were performed using the SPSS statistical software package (Version 11.0; SPSS, Chicago, IL). SAS software was used for statistical analysis of comet assay data. p -Value less than 0.05 was considered statistically significant.

Results

HDTIC-1 and HDTIC-2 slowed down telomere shortening of 2BS cells

The telomere lengths of the 2BS cells treated with HDTIC are shown in Figure 2 and Table 1. Telomeres of 2BS cells grown from PD28 in 0.1 μ M HDTIC-1 or 1.0 μ M HDTIC-2 were shortened by 31.5 bp per PD or 41.1 bp per PD, respectively. In contrast, telomere shortening rate of the control cells was 71.1 bp per PD. At PD55, when telomere length in control cells reached the minimum (7.01 kb), the treated cells retained telomeres 1.07 kb (0.1 μ M HDTIC-1 treatment) and 0.81 kb (1.0 μ M HDTIC-2 treatment) longer than those of the control cells. These results showed that the two isomers of HDTIC significantly slowed down the shortening rate of telomere of 2BS cells. The cells cultured with HDTIC-1 at the final PD74 showed a TRF of 6.97 kb, and the cells cultured with HDTIC-2

at the final PD69 had a TRF of 6.86 kb, which was similar to that (7.01 kb) of the control cells at last PD.

HDTIC-1 and HDTIC-2 protected genomic DNA from oxidative damage induced by H₂O₂

The single-strand breakages of DNA of PD55 2BS cells grown from PD28 in DMEM supplemented with or without HDTIC were measured with comet assay. The results showed that most of the control cells and HDTIC-cultured cells at late passage had no obvious comet tail (data not shown).

The protection of genomic DNA of 2BS cells by HDTIC from oxidative stress induced by 200 μ M H₂O₂ was analyzed using comet assay (see Table 2, Fig. 3, Fig. 4). PD28 2BS cells, pretreated with HDTIC-1 or HDTIC-2 for 12 h, were exposed to 200 μ M H₂O₂ for 5 min in a dark. The HDTIC-pretreated cells had a significant reduction in DNA damage. Moreover, the extent of DNA damage decreased significantly when the concentrations of HDTIC increased. The cells pretreated with 1.0 μ M HDTIC-1 or 10.0 μ M HDTIC-2 had no obvious comet tail after exposure to 200 μ M H₂O₂ for 5 min, while the comet tail length and the comet tail area percentage of the control cells were 82.5 μ m and 70.8%, respectively.

TABLE 2. THE DEGREE OF THE 200 μ M H₂O₂-INDUCED DNA DAMAGE OF THE POPULATION DOUBLING (PD28) 2BS CELLS PRETREATED WITH DIFFERENT CONCENTRATIONS OF HDTIC FOR 12 H

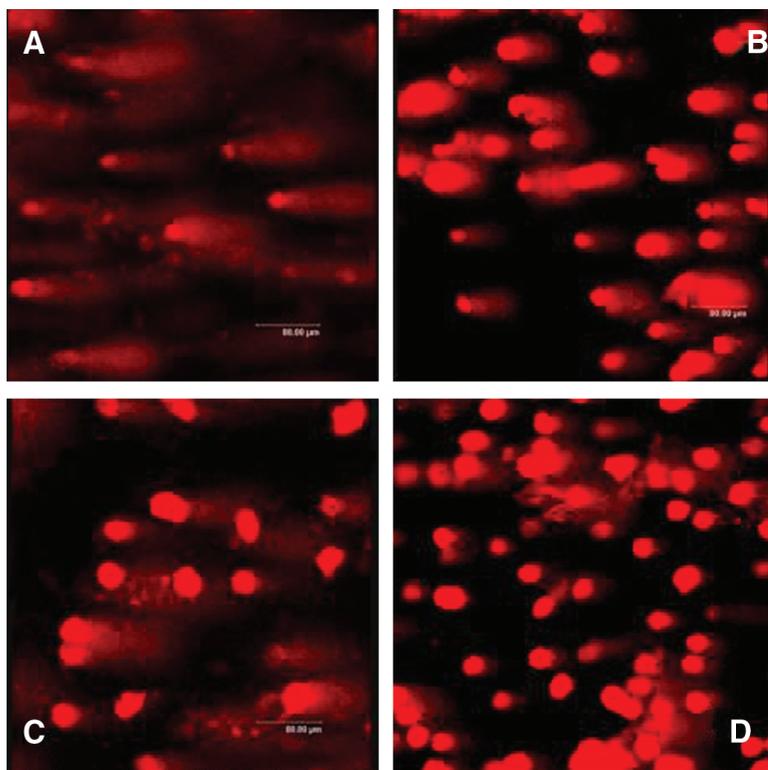
Group	Tail length (μ m)	Tail area/total area (%)
Control	122.42 \pm 6.21	92.12 \pm 3.25
0.1 μ M HDTIC-1	80.57 \pm 5.44 ^a	70.82 \pm 7.51 ^b
0.2 μ M HDTIC-1	39.55 \pm 3.06 ^a	34.25 \pm 5.86 ^b
1.0 μ M HDTIC-1	24.78 \pm 5.15 ^a	21.38 \pm 1.13 ^b
1.0 μ M HDTIC-2	81.83 \pm 9.37 ^a	73.14 \pm 6.34 ^b
2.0 μ M HDTIC-2	51.41 \pm 4.74 ^a	50.77 \pm 6.66 ^b
10.0 μ M HDTIC-2	11.50 \pm 2.82 ^a	10.52 \pm 1.68 ^b

PD28 2BS cells were cultured with Dulbecco's modified Eagle's medium containing different concentrations of HDTIC-1 or HDTIC-2 for 12 h. The cells were damaged with 200 μ M H₂O₂ for 5 min in the dark. Then the cells were washed and harvested with PBS to analyze DNA damage using comet assay. One hundred cells were counted for each sample to measure the average values of comet tail length and comet tail area.

Compared with control, ^a $p < 0.05$ and ^b $p < 0.05$.

PBS, phosphate-buffered saline.

FIG. 3. The protective effects of different concentrations of HDTIC-1 on genomic DNA of PD28 2BS cells against oxidative stress induced by H_2O_2 . PD28 2BS cells were cultured with Dulbecco's modified Eagle's medium (DMEM) containing different concentrations of HDTIC-1 for 12 h. Then, the cells were exposed to $200 \mu M H_2O_2$ for 5 min at $4^\circ C$ in the dark. DNA damage was analyzed using comet assay. The pictures were taken at $600\times$ magnification using a fluorescence microscope. (A) Control 2BS cells; B, C, and D represent 2BS cells pretreated with 0.1, 0.2, and $1.0 \mu M$ HDTIC-1, respectively. Color images available online at www.liebertonline.com/dna.



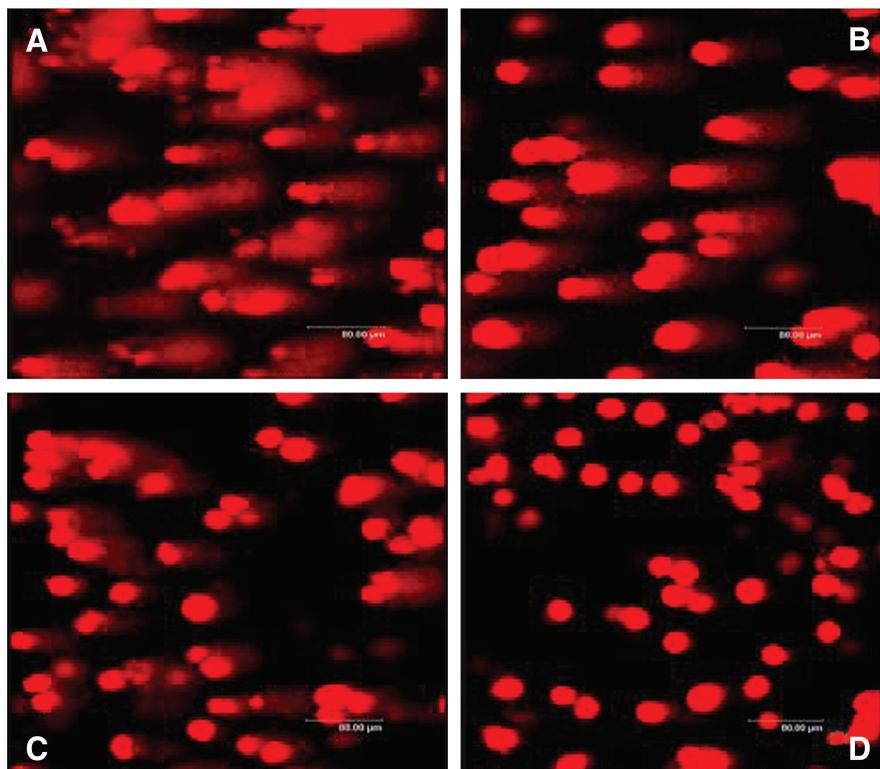
HDTIC compounds improved the ability of 2BS cells to repair oxidative DNA damages

PD31 2BS cells, predamaged by $100 \mu M H_2O_2$ for 5 min in the dark, returned to normal phenotypes when the damaged

cells were treated with $1.0 \mu M$ HDTIC-1 or $10.0 \mu M$ HDTIC-2 for 1 h, while the control cells remain seriously damaged even after they were cultured in normal DMEM for 1 h (Fig. 5).

The result of comet assay showed that most of the damaged cells after HDTIC treatment had no obvious comet tails,

FIG. 4. The resistance of PD28 2BS cells pretreated with HDTIC-2 to the damage induced by H_2O_2 . PD28 2BS cells were cultured with DMEM containing different concentrations of HDTIC-2 for 12 h. Then, DNA damage was analyzed using comet assay after the cells were exposed to $200 \mu M H_2O_2$ for 5 min at $4^\circ C$ in the dark. The pictures were taken at $600\times$ magnification using a fluorescence microscope. (A) Control 2BS cells; B, C, and D represent 2BS cells pretreated with 1.0, 2.0, and $10.0 \mu M$ HDTIC-2, respectively. Color images available online at www.liebertonline.com/dna.



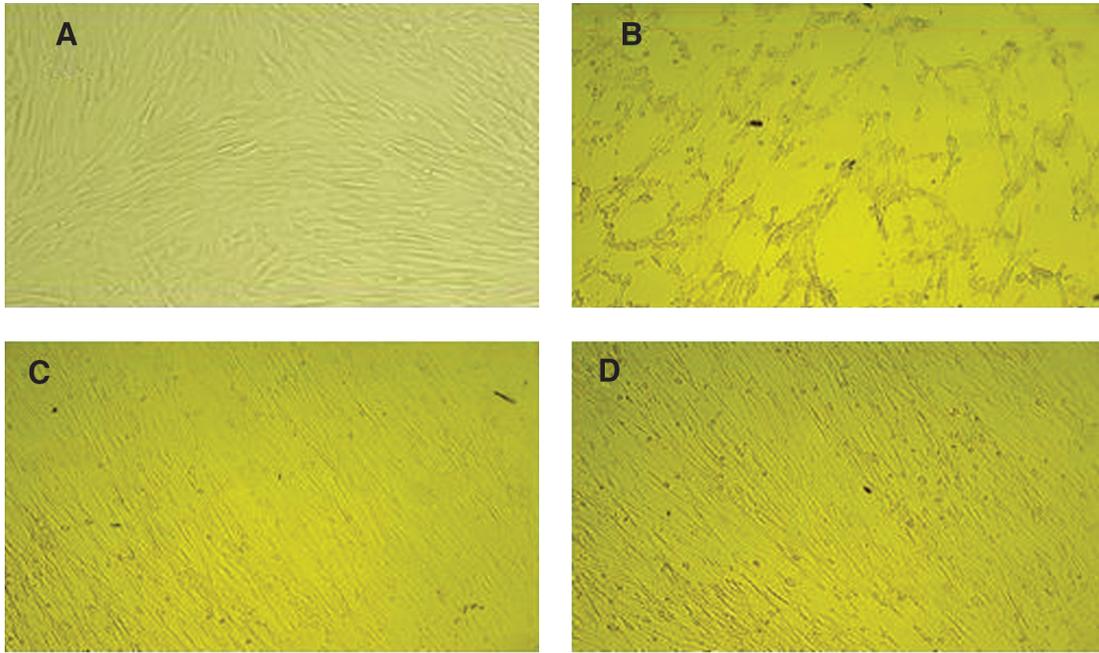


FIG. 5. The rescuing potential of HDTIC compounds against H₂O₂-induced premature death or damage in 2BS cells (original magnification 100×). PD31 2BS cells (A) were damaged by 100 μM H₂O₂ in the dark for 5 min. The 2BS cells were washed twice with PBS and then continually cultured with normal DMEM (B), or the DMEM containing 1.0 μM HDTIC-1 (C) or 10 μM HDTIC-2 (D) for 1 h. Color images available online at www.liebertonline.com/dna.

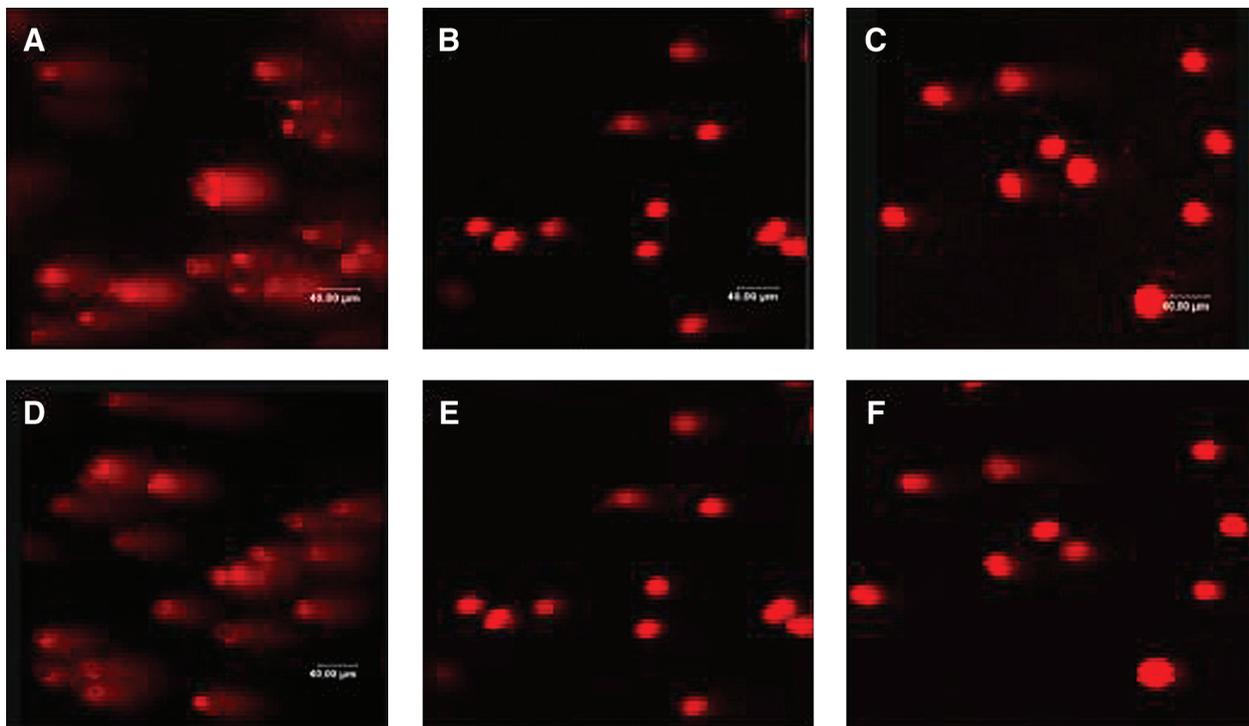


FIG. 6. The effect of HDTIC on the DNA repair ability of 2BS cells. PD31 2BS cells were damaged by 100 μM H₂O₂ in the dark for 5 min and continually cultured with DMEM supplemented with 0 μM HDTIC-1 (A), 0.1 μM HDTIC-1 (B), 1.0 μM HDTIC-1 (C), 0 μM HDTIC-2 (D), 1.0 μM HDTIC-2 (E), or 10 μM HDTIC-2 (F) for 1 h. DNA damage was analyzed by comet assay. Color images available online at www.liebertonline.com/dna.

TABLE 3. THE EFFECTS OF HDTIC COMPOUNDS ON THE DNA REPAIR ABILITY OF 2BS CELLS EXPOSED TO OXIDATIVE STRESS

Group	Tail length (μm)	Tail area/total area (%)
Control	109.31 \pm 13.26	84.07 \pm 10.01
0.1 μM HDTIC-1	16.82 \pm 2.10 ^a	24.39 \pm 2.71 ^b
1.0 μM HDTIC-1	11.67 \pm 1.46 ^a	18.25 \pm 2.61 ^b
1.0 μM HDTIC-2	19.08 \pm 2.73 ^a	20.84 \pm 2.98 ^b
10.0 μM HDTIC-2	9.93 \pm 1.42 ^a	12.84 \pm 1.83 ^b

PD31 2BS cells were damaged with 100 μM H_2O_2 for 5 min in the dark. Then the cells were washed with PBS and continually cultured with Dulbecco's modified Eagle's medium supplemented with or without HDTIC for 1 h. Then the cells were harvested with PBS to analyze DNA damage using comet assay. One hundred cells were counted for each sample to measure the average values of comet tail length and comet tail area.

Compared with control, ^a $p < 0.001$ and ^b $p < 0.001$.

whereas the control cells without HDTIC treatment had long comet tails (109.3 μm of the tail length and 84.1% of the tail area in average) (see Table 3, Fig. 6). These results indicated that 100 μM H_2O_2 -induced DNA damages could be rapidly repaired in HDTIC-supplemented DMEM, suggesting that HDTIC compounds significantly improved the ability of 2BS cells to repair DNA.

Discussion

A telomere, the tandem repeat arrays of the hexamer sequence (TAAGGG) $_n$, is the natural end of chromosomes. It helps to stabilize the chromosome and is destined to shorten during aging or replicative senescence (Kipling, 2001). For example, the telomeres of human fibroblasts shorten by 50–100 bp with each division (Harley *et al.*, 1990; Allsopp *et al.*, 1992). Similar observations have been made in other cultured human cell types (Chang and Harley, 1995; Bodnar *et al.*, 1998). When the telomeres of normal fibroblasts reach a limited length, the cells lose the potential to divide and arrest in G0 or G1 phase. In fact, the erosion of telomere has early been suspected and later proven as a biological clock in proliferating fibroblasts (Harley *et al.*, 1990; Bodnar *et al.*, 1998). It is now regarded as an important trigger of replicative senescence. The lifespan of fibroblasts or the speed of replicative senescence depends mainly on the shortening rate of telomere.

In this study, we demonstrated that HDTIC-1 and HDTIC-2 were able to slow down the shortening rate of telomeres in 2BS cells by 56% and 42%, respectively, which satisfactorily explains the fact that the two compounds can obviously extend the lifespan of 2BS cells (Wang *et al.*, 2003). In addition, the loss of telomeric repeats might induce a corresponding check point response and influence the silencing of cell-cycle genes located in the vicinity of telomeres (Vijg, 2008). We speculate, therefore, that the slow down of telomere shortening caused by HDTIC-1 and HDTIC-2 may be responsible for their promotion of the entry of 2BS cells from G₀ or G₁ phase to S phase (Wang *et al.*, 2003). 2BS cells treated with 0.1 μM HDTIC-1 from PD28 showed a TRF of 6.97 kb at the final PD74, and the cells cultured with 1.0 μM HDTIC-2 at the final PD69 had a TRF of 6.86 kb, which was similar to that (7.01 kb) of the control cells at last PD. These results suggest

that the shortest length of telomere that is necessary for continuation of cell division is independently settled.

There are different causes for telomere shortening with replicative age; however, the major determinant of telomere shortening rate in human diploid fibroblasts under normal culture conditions appears not to be an end replication problem but a telomere-specific accumulation of DNA single-strand damage caused by oxidative stress (Proctor and Kirkwood, 2002; von Zglinicki *et al.*, 2000). If the DNA single-strand breaks occur closely to the end of a chromosome, they might transiently stall the replication fork or inhibit the formation of an Okazaki fragment opposite to the break and, eventually, lead to the distal fragment remaining unrepliated. The assumption above is strongly supported by the fact that telomeres are lost 5 to 10 times faster than normal if fibroblasts are subjected to H_2O_2 , chronic hyperoxia, etc. On the other hand, the rate of telomere erosion decreases by half or more if the cells are treated with free radical scavengers, such as α -phenyl-t-butyl-nitron, vitamin C, etc. (von Zglinicki *et al.*, 1995; von Zglinicki *et al.*, 2000; Wang *et al.*, 2007; Aubert and Lansdorp, 2008).

Therefore, reducing oxidative stress and increasing the ability to repair DNA damage can slow down telomere shortening rate. In this study, we showed that PD28 2BS cells, pretreated with HDTIC-1 or HDTIC-2 for 12 h, had a significant reduction in DNA single-strand breaks after exposure to 200 μM H_2O_2 for 5 min. This reduction in DNA damage appeared more obvious when the concentrations of HDTIC increased. These results suggest that HDTIC-1 and HDTIC-2 significantly improve the antioxidative potential and offer an obvious protection of 2BS cells from stress-induced DNA damage. Moreover, when the cells damaged by 100 μM H_2O_2 were incubated with HDTIC for 1 h, the damaged cells returned to normal both in size and in morphology. 100 μM H_2O_2 -induced DNA damage was greatly reduced by 1.0 μM HDTIC-1 or 10 μM HDTIC-2, indicating that HDTIC compounds strongly improve the ability of 2BS cells to repair DNA. The observation that HDTIC increases the antioxidative potential and the DNA repair ability satisfactorily explains how HDTIC slows down telomere shortening.

DNA damage leads to error of protein expression or no expression. Damage to DNA-binding domains can not only block the binding of transcription factors to the relevant domains but also cause cell growth arrest through inducing p53 (Chen and Ames, 1994; Di Leonardo *et al.*, 1994; Smith and Fornace, 1996). Also, p16, the senescence-associated gene (Kim and Sharpless, 2006), was demonstrated to be down-regulated by HDTIC in our previous study (Wang *et al.*, 2008). We hypothesized that HDTIC compounds influence the senescence-associated pathway by reducing DNA damage.

In summary, HDTIC-1 and HDTIC-2 can significantly slow down telomere shortening in 2BS cells, which can be mainly attributed to their antioxidative potentials and DNA repair ability. In addition, the slow down of telomere shortening rate, the reduction of DNA damage, and the improvement of DNA repair ability induced by HDTIC may be responsible for their delay of replicative senescence.

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Disclosure Statement

There are no competing financial interests with regard to this work.

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