CELL CYCLE
2022, VOL. 21, NO. 14, 1519–1531
https://doi.org/10.1080/15384101.2022.2055252

RESEARCH PAPER

Intestinal aging is alleviated by uridine via regulating inflammation and oxidative stress *in vivo* and *in vitro*

Na Jiang and Zhiwei Zhao

Department of Gastroenterology, Wuhan Hankou Hospital, Wuhan, Beijing, China

ABSTRACT
Many countries in the world are stepping into the aging society with the challenge of the increasing agin gpopulation. The physiological functions of the human body begins to decline with aging, and the intestinal tract as the most important digestive organ will also be aging. How to relieve or reverse aging is an important scientific problem.The aging model *in vivo* and *in vitro* was established. Western-blot, indirect immunofluorescence and immunohistochemistry were carried out to explore the anti-aging effect of uridine.In the current study, we examined the anti-aging effect of uridine *in vivo* and *in vitro* experiments. *In vitro* cell model, we found that the aging level of intestinal tract was significantly reduced by uridine, uridine treatment down-regulated the Sa-β-gal-positive cells. Furthermore, the levels of inflammation and oxidative stress were also significantly reduced by uridine treatment. On this basis, *in vivo* experiments, we found that the aging level of mice fed with uridine was significantly lower than that of the control group as demonstrated by immunohistochemistry and Western blot analyses.In conclusion, our current research indicates that uridine shows a good anti-aging effect,which suggests that uridine is expected to be used as a health food or clinical drug to treat intestinal aging.

ARTICLE HISTORY
Received 14 December 2021
Revised 13 February 2022
Accepted 09 March 2022

KEYWORDS
Uridine; aging; intestinal tract; inflammation

Introduction

With the coming of boom in aging population, many countries in the world have begun to enter the aging society [1]. The physiological function of the body begins to decline gradually with aging, the body's tissues and organs will also be aging and functional decline [2]. Gastrointestinal tract is the main place for the body to absorb nutrition, therefore intestinal tract is an important target organ for the body to prolong life. Intestinal aging can lead to many negative changes in intestinal tissue structure and function. The gut is the most important place to absorb nutrients [3], the aging of intestinal tract will lead to a series of negative effects on other organs and tissues. Intestinal aging could lead to structural changes and mucosal barrier dysfunction, increase oxidative stress, decrease protective immunity, which seriously affects the quality of life and health of the elderly [4]. At the same time, it can directly or indirectly cause and aggravate the occurrence and development of some organic diseases, such as gastrointestinal tumors and cardiovascular and cerebrovascular accidents [4].

Uridine is mainly derived from animals or plants, and it has been reported that uridine is decreased with aging [5]. As a pyrimidine nucleoside, uridine provides the material basis for a variety of metabolic processes. Uridine is an important component of cell nucleic acid. Previous studies showed that uridine can improve the immunity ability [6]. Animal studies have shown that the combination of uridine and inosine could promote myocardial cell metabolism, accelerate protein and nucleic acid biosynthesis and energy production, and promote and improve brain cell metabolism [7]. In addition, it has been reported that uridine has the cardioprotective effect [8]. Furthermore, uridine can be used to treat NRTI-related mitochondrial toxicity [9,10]. Additionally, recent study showed that uridine has pharmacological effects, for example, Zheng et al reported that uridine could alleviate CCL4-induced liver fibrosis [11]. Most recently, Ye et al

CONTACT Na Jiang jiangnana11@126.com Department of Gastroenterology, Wuhan Hankou Hospital, Wuhan, Beijing, 430000 China
Supplemental data for this article can be accessed here
© 2022 Informa UK Limited, trading as Taylor & Francis Group

found that uridine has the great potential in the treatment of osteoarthritis (OA) [12].

In the current study, we evaluate the anti-aging effect of uridine using intestinal cells as an *in vitro* model and aged mice as an *in vivo* model. We found that uridine had anti-aging effect *in vivo* and *in vitro*, which reduced the rate of β-gal-positive cells, reduced the expression of inflammatory-related cytokines and the levels of oxidative stress. *In vivo* experiments, uridine also alleviated the aging level of intestinal tract of the aged mice. Taken together, the current study shows that uridine exhibits a good anti-aging effect. This work indicates that uridine is expected to be used as a health food or clinical drug to treat intestinal aging.

Materials and methods

Antibodies and reagents

P16 (#ab211542,1:1000 dilution), P21 (#ab109199, 1:2000 dilution), NFκB (#ab28849, 1:1500 dilution), P38 (#ab195049, 1:1000 dilution), Occludin (#ab21-6327. 1:1500 dilution), claudin4 (#ab15104,1:1000) and ZO-1 (#ab216880, 1:2000 dilution) antibodies were purchased from Abcam company (UK). Uridine was purchased from Sigma-Aldrich (USA). BCA Protein Concentration Kit, RIPA lysate, glutathione peroxidase (GSH-PX) assay kit, reactive oxygen assay species assay kit, MDA assay Kit and Cell superoxide dismutase (SOD) assay kit were obtained from Biyuntian biotechnology company (Shanghai, China). DMEM culture medium and Fetal bovine serum (FBS) were obtained from Thermo Fisher (USA). Goat anti-mouse IgG secondary antibody were purchased from CST company (Cell Signaling technology). An enhanced chemiluminescence (ECL) kit was purchased from Merck Millipore. Mouse IL-6 ELISA Kit (ab222503), Mouse TNFα ELISA Kit (ab208348) and Mouse IL-1 beta ELISA Kit (ab19-7742) were purchased form Abcam (Cambridge,UK). Polyvinylidene fluoride (PVDF) membrane was purchased from Millipore (Massachusetts, USA). The reverse transcription kit was purchased from Applied Biosystems (Foster City, CA, USA). Unless otherwise specified, other reagents were purchased from Sigma-Aldrich.

IEC6 and FHs 74 int cell culture

IEC6 cell and FHs 74 Int were purchased from the American Type Culture Collection (ATCC). IEC6 and FHs 74 Int cells were cultured in DMEM medium containing 10% FBS, 1% penicillin/streptomycin at 37°C in a humidified 5% CO_2 (vol/vol) atmosphere.

Animal care

Female C57BL/6 mice (young group: 5 months; aged group: 21 months) were purchased Huafukang biotechnology Co., Ltd. Animals (3–5 animals/cage) were housed in temperature-controlled (22°C) and humidity-controlled (45%) rooms under a 12:12-h light–dark cycle, mice had ad libitum access to food and water. The animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the ethical committee of Han-Kou Hospital (Wu Han).

Laser scanning confocal microscope (CLSM)

IEC6 cell samples were washed with PBS for three times. The cell samples were then blocked with 5% BSA. After three washes, the cells were incubated for 24 h with the indicated antibodies at 4°C. After three washes, fluorescently-labeled secondary antibody was then added and incubated for 1 h at 37°C. Hoechst was used to stain the cell nuclei. After the cells were washed with PBS for 3 times, the cell samples were observed by using CLSM (FV3000).

Western-blot

The proteins from tissue or cells were extracted using the lysis buffer. The extracted total protein was then measured by BCA Protein Assay Kit. 30 μg total protein (each lane) was subjected to SDS-PAGE, and then transferred to the PVDF membrane. After three washes with TBST, the membranes were incubated with the primary antibody in 5% BSA/PBS/Tween at 4°C overnight. The cell samples were washed and incubated with the secondary antibody for 2 h at RT. Enhanced chemiluminescence (ECL) solution was

used to detect protein bands. Quantification of corresponding protein expression levels was normalized to controls. Image J software was used to conduct semi-quantitative analysis of blots.

MTT assay

MTT assays were used to detect the effect of uridine on intestinal cell proliferation. Cells in logarithmic growth phase were seeded in 96-well cell plates at a density of 1×10^4 cells/ml (100 µl per well), and cultured in 5% CO_2 incubator at 37°C. After the cells were adhered to the plate, different concentrations of uridine were added and incubated at different time points (24–72 h). The culture medium was then discarded, and 100 µl of 0.5 mg/mL MTT solution was added into each well, and the absorbance value was measured in an ELISA reader at 450 nm.

RT-PCR

Total RNA was extracted from intestinal cells or mice tissues by using RNAzol kit according to the manufacturer's instructions. Then RNA was then reverse-transcribed into complementary DNA (cDNA) using a TaKaRa RNA PCR Kit (TaKaRa, Shiga, Japan). The quantitative RT-PCR was carried out with a total reaction volume of 20 µl PCR reaction system, including Master mix (10 µL), upstream and downstream primers (0.5 µL), cDNA template (2 µL), ROX reference dye (1 µL), ddH_2O (6 µL). Amplification conditions were as follows: predenaturation at 95°C for 5 min, denaturation at 95°C for 15s, annealing/extension at 65°C for 30s for 40 cycles. The relative expression of inflammatory factors was calculated by $2^{-\Delta\Delta}$CT method. The primer sequence: IL-1β: F:CAACCAA CAAGTGATATTCTCCATG,R:GATCCACACTCT CCAGCTGCA; IL-6: F: GAGGATACCACTCCCA ACAGACC, R: AAGTGCATCATCGTTGTTCATA CA; TNFα: F:CATCTTCTCAAAATTCGAGTGA CAA, R:TGGGAGTAGACAAGGTACAACCC.

HE staining

The intestinal tissue was washed with PBS and fixed in 4% PFA solution. After the tissue samples were dehydrated, they were routinely made into paraffin sections, and tissue staining was then performed using a HE staining kit (Beyotime, Hangzhou, China) according to the manufacturer's instruction.

Immunochemistry

The slice thickness of the intestinal tissue was 4 µm. The sections were deparaffinized with xylene. The samples were rehydrated to water through a graded series of ethanol concentrations, and washed in running water. After rinsing the samples with running water and phosphate-buffered saline (PBS) solution (pH = 7.4) for three times (3 min each time). The tissue sections were boiled at 110°C for 20 min for heat-induced antigen retrieval. The samples were then rinsed with running water and PBS solution 3 times for 3 min each time. 3% H_2O_2 was added and incubated for 10 min at room temperature in the dark, after which, the sections were rinsed with PBS solution for three times (3 min each time). Primary antibody was added and incubated overnight at 4°C. After washing the sample with PBS solution, secondary antibody was added for staining. The sample was observed under a microscope.

ELISA assay

The levels of IL6, IL-1βand TNFα were detected by ELISA Kits according to the manufacturer's instructions.

Flow cytometry analysis

The cells were digested using 0.25% trypsin and collected by centrifugation, the supernatant was removed and cell pellets were re-suspended. After the cells were fixed and blocked, the indicated primary antibody was added and incubated for 2 h. After washing, the fluorescently-labeled secondary antibody was added and incubated for 1 h. The cells were then washed three times, and the cell samples were detected by Flow cytometer (BD Accuri C6 Plus).

Statistical analysis

Data were analyzed using Graph Pad Prism version 9.0. All results are expressed as mean ± standard error of mean. SPSS23.0 software was used

for single-factor analysis of variance (one-way ANOVA), and statistical significance was set at $P < 0.05$.

Results

Establishment of the model of IEC6 senescence by H_2O_2

In the current study, we established the senescent IEC6 model by H_2O_2 treatment, IEC6 cells were exposed to different concentrations of H_2O_2 (10–50 μmol/L) to induce IEC6 senescence for 2.5 h, after which, the culture medium was replaced with fresh medium, and cells were cultured for another 24 h. IEC6 cells were then collected for assessment of cell senescence. The results showed that H_2O_2 (20 μmol/L) could induce IEC6 senescence which was confirmed by the following experiments: 1) Sa-β-gal (senescence-associated beta-galactosidase) staining showed that H_2O_2 treatment induced IEC6 cell senescence (Figure 1a), Flow cytometry showed that H_2O_2 (20 μmol/L) did not lead to significant cell apoptosis compared to control group (Figure 1b); 2) The expression level of cell senescence markers (P15 and P16) was significantly up-regulated (Figure 1c); 3)

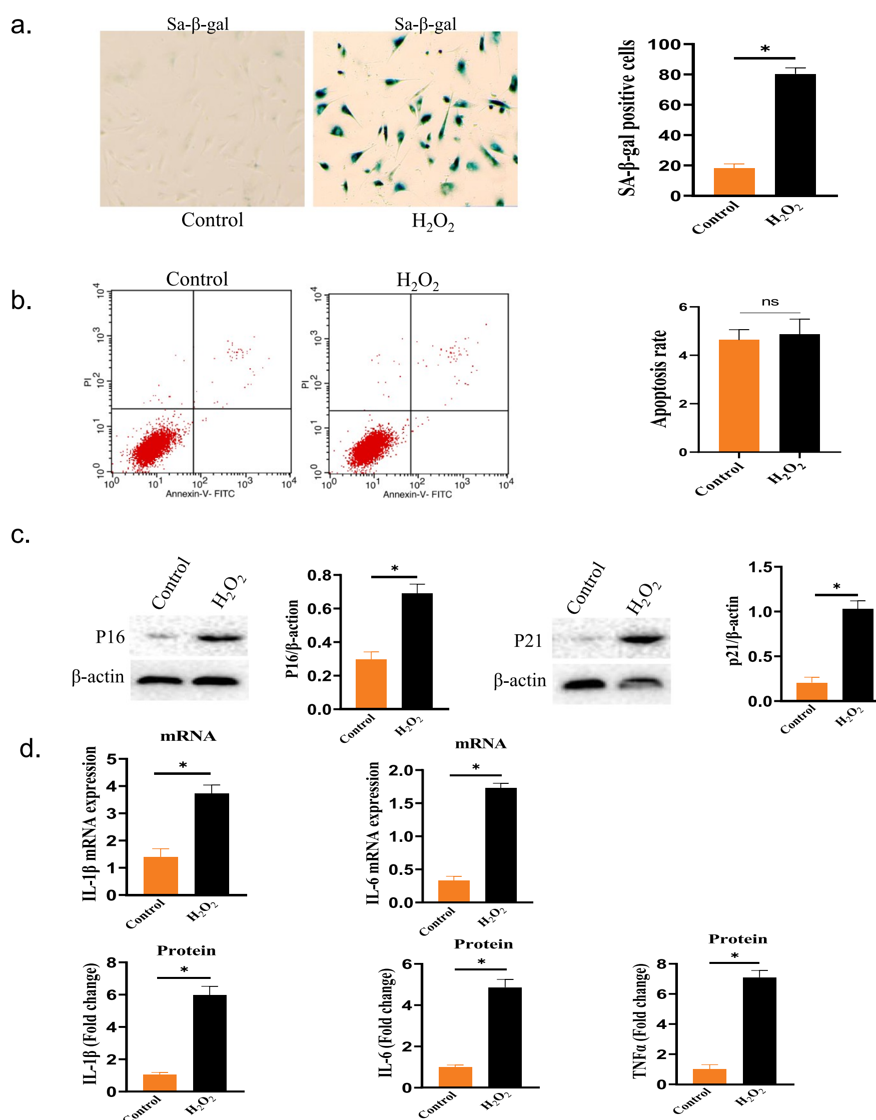

Figure 1. a. Senescent IEC6 cell model was established by H_2O_2 treatment. b. H_2O_2 did not induce IEC6 apoptosis by Flow cytometry analysis; c. H_2O_2 induced the expression of P21 and P16. d. The mRNA of IL-1β and IL-6 expression were increased in the senescent IEC6, n = 3. e. The cellular senescence model of FHs 74 (int intestinal cell line) was established. f. Evaluation of cell cycle and cell apoptosis.The asterisk indicates a significant difference ($p < 0.05$).

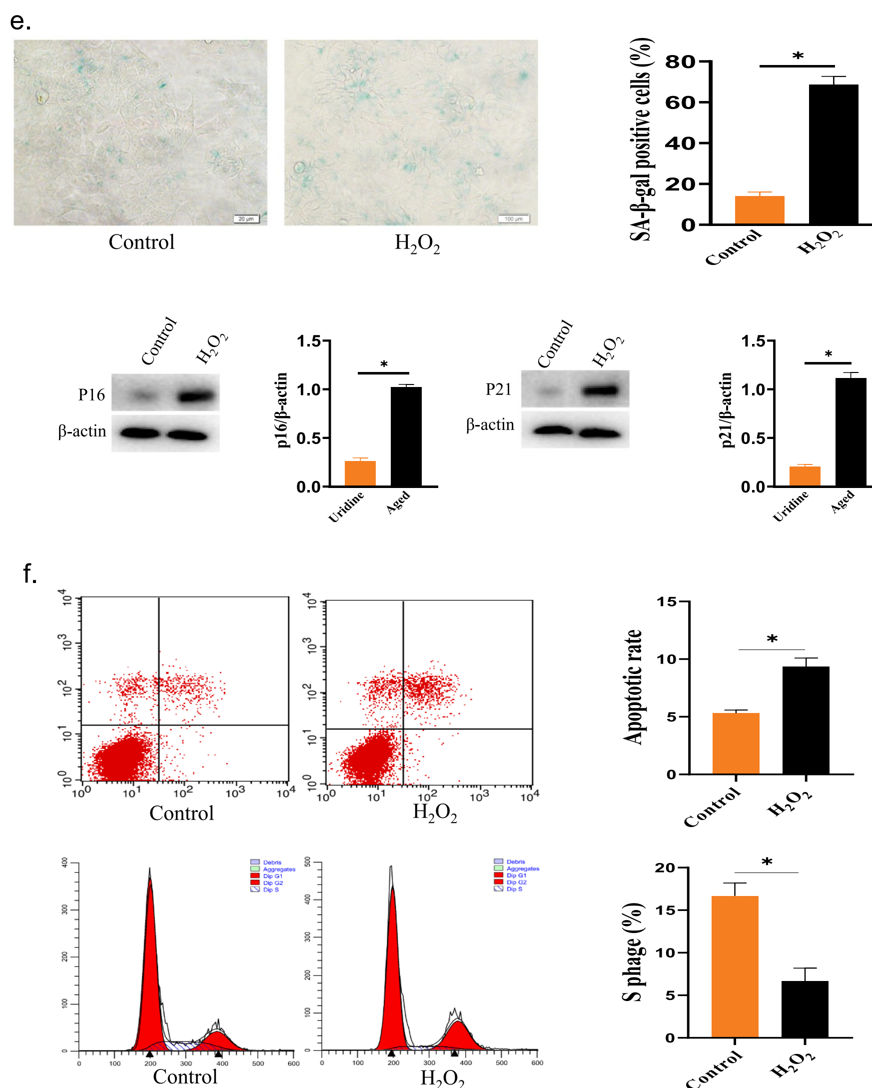

Figure 1. (Continued).

The cell senescence may be closely related to inflammation. Therefore, the expression of the proinflammatory molecules were also checked, and the results showed that the mRNA or proteins expression of interleukin-6 (IL)-6 and IL-1β were also up-regulated (Figure 1d). In addition, we also established another cellular senescence model using intestinal cell line (FHs 74 Int). The results showed that H_2O_2 (30 μmol/L) could induce FHs 74 Int senescence (Figure 1e), and ell cycle analysis showed that the proportion of S phase was significantly reduced in the H_2O_2 treatment group, and the apoptosis rate was slightly increased in the H_2O_2 treatment group (Figure 1f).

Uridine could alleviate the intestinal cell senescence

Here, we tested the effect of uridine on the intestinal cell senescence. As shown in Figure 2a, uridine significantly alleviated IEC6 senescence. The results of Sa-β-gal staining showed that the rate of β-gal positive cells was significantly reduced by uridine treatment. Furthermore, the expression level of cell senescence markers (P15 and P16) was significantly down-regulated (Figure 2b). In addition, the nuclear size and LAP2 (Leucine aminopeptidase) intensity between control group and uridine-treated group were also analyzed, and the results also showed that uridine significantly inhibited the IEC6 senescence

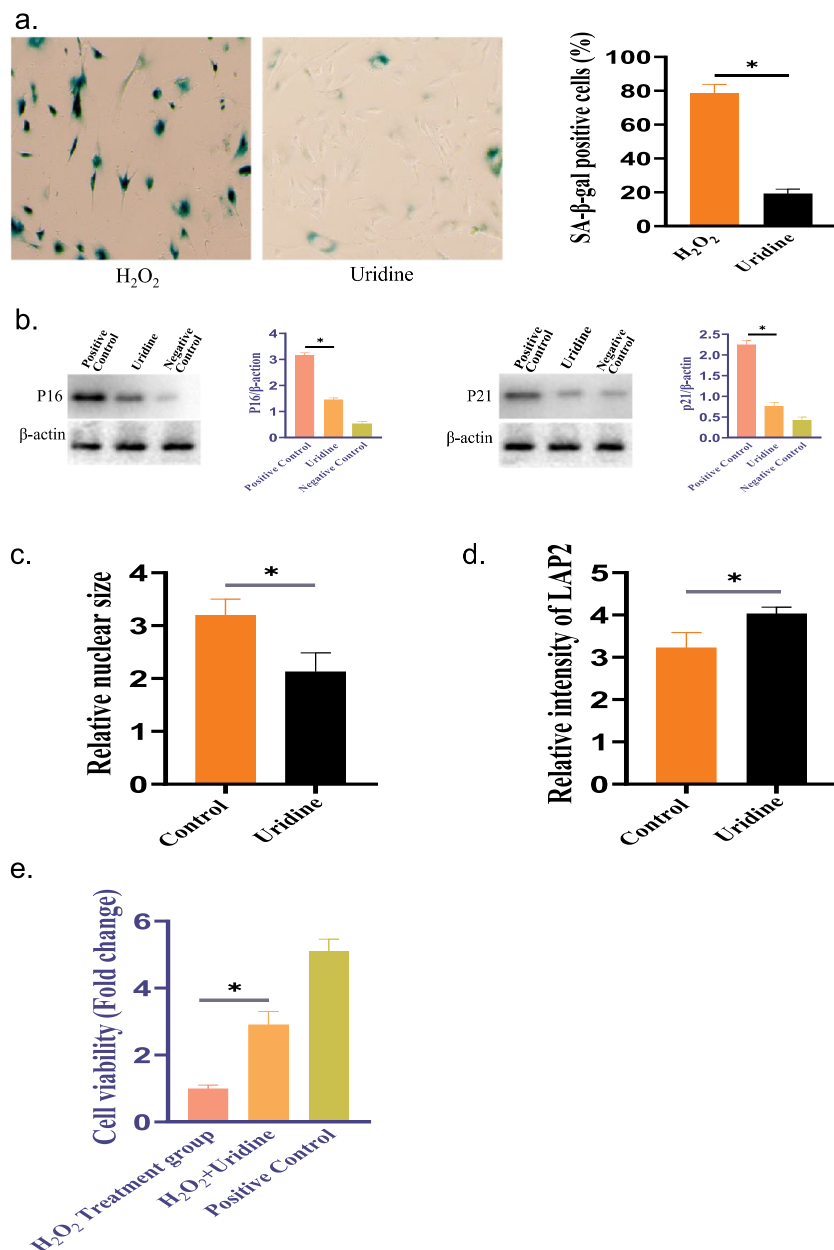

Figure 2. a. β-gal positive cells was significantly reduced by uridine treatment. b. Uridine down-regulated P16 and P21 in the senescent IEC6. c-d. Detection of the nuclear size and LAP2 expression. e. The effect of uridine on cell proliferation (n = 5). f. The effect of uridine on senescence of intestinal cell line (FHs 74 Int) by Sa-β-gal and Western-blot assays. g. Evaluation of cell cycle and cell apoptosis. Results were presented as means ± SD. The asterisk indicates a significant difference ($p < 0.05$).

(Figure 2c,d). Cell proliferation assay results indicated that the cell proliferation ability in the uridine-treated group was significantly enhanced compared with the control group (Figure 2e).

Additionally, we also tested the effect of uridine on cell senescence in the intestinal cell line (FHs 74 Int), we also found that uridine obviously mitigated the senescence of FHs 74 Int cell by Sa-β-gal and Western-blot assays (Figure 2f). Furthermore, the cell cycle (S phase) was partially rescued, and the cell apoptotic rate was also significantly down-regulated after uridine treatment (Figure 2g).

Next, we evaluated the effect of uridine on the expression of the pro-inflammatory cytokines, and found that the mRNA or proteins expression levels of interleukin-6 (IL-6) and IL-1β were down-regulated (Figure 3a). We further analyzed the potential molecular mechanism by which uridine could reverse inflammation. As

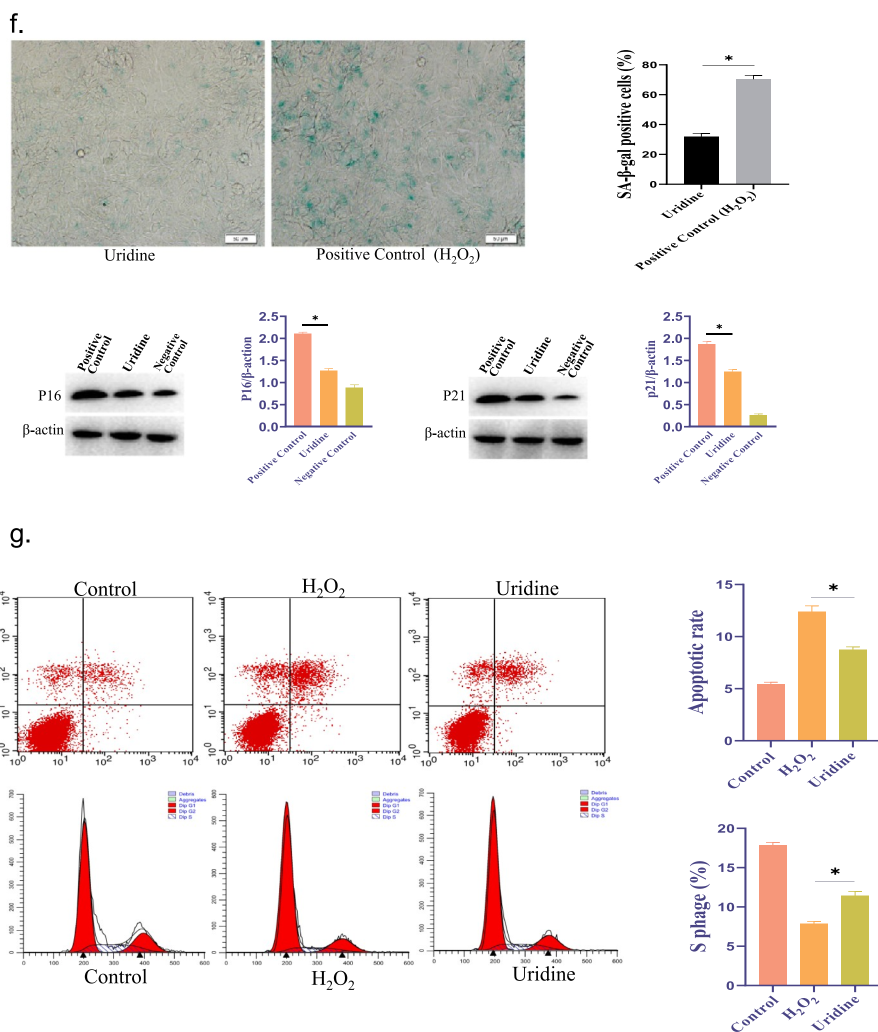

Figure 2. (Continued).

shown in Figure 3b, the inflammatory-related signaling pathways (NFκB, p38, MAPK and JUK) were down-regulated by uridine treatment.

Effect of uridine on oxidative stress

Aging is closely related to oxidative stress. H_2O_2 also can induce oxidative stress, and oxidative stress is also closely related to cell senescence. As expected, we found that the level of oxidative stress was up-regulated in senescent IEC6 cells. Therefore, we further tested the effect of uridine on oxidative stress in senescent IEC6 cells, it can be seen that uridine attenuated the oxidative stress of the senescent IEC6 as determined by oxidative stress markers (such as ROS) and anti-oxidative stress markers (such as SOD) (Figure 4), suggesting that uridine has anti-oxidative stress effect.

Effect of uridine on intestinal barrier

It is well-known that aging is closely associated with the integrity of intestinal barrier [13]. Therefore, the integrity of intestinal epithelial barrier is easily damaged with aging. Therefore, in the current study, we preliminarily evaluated the effect of uridine on tight junction-associated proteins (occludin, claudin4, and ZO-1).Western blot results showed that the protein expression of occludin, claudin4, and ZO-1 was down-regulated in senescent IEC6 and FHs 74 Int cells after uridine treatment (Figure 5). However, the

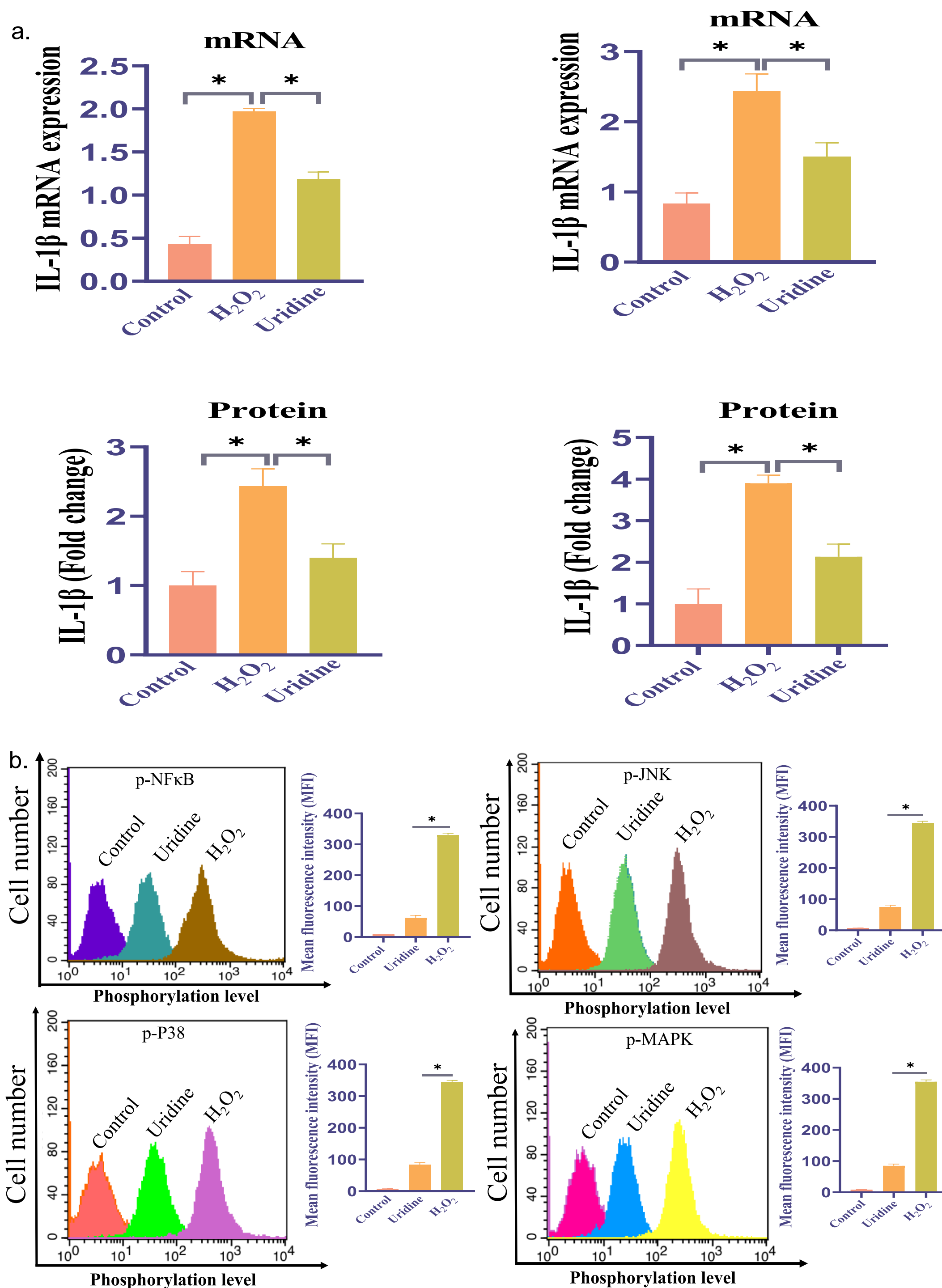

Figure 3. A. IL-6 and IL-1β were down-regulated by uridine treatment. B. The inflammatory signaling pathways were down regulated under uridine treatment. The total protein was extracted. Each sample (30 μg) was subjected to SDS-PAGE, and then transferred to the PVDF membrane. After three washes with TBST, the membranes were incubated with the primary antibody at 4°C overnight. The cell samples were washed and incubated with the secondary antibody for 2 h at RT. Enhanced chemiluminescence (ECL) solution was used to detect protein bands (n = 5). Results were presented as means ± SD. The asterisk indicates a significant difference ($p < 0.05$).

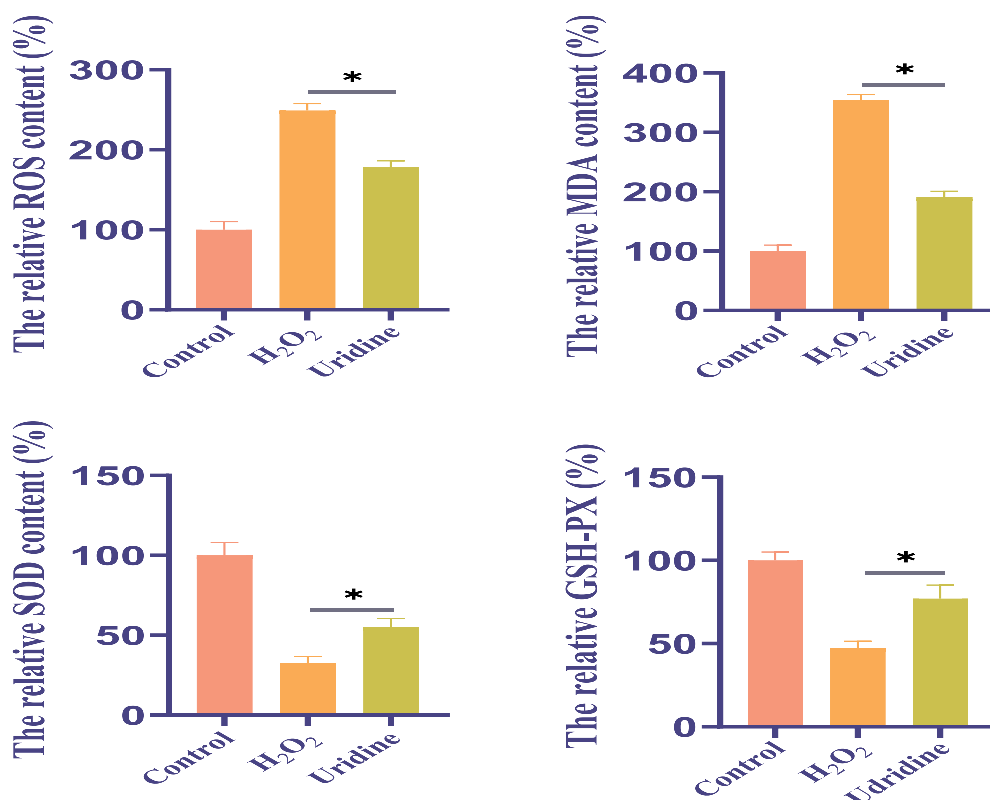

Figure 4. Uridine attenuated the oxidative stress of the senescent IEC6 cells. The detailed process of the experiment has been described in detail in the Materials and Methods section. The asterisk indicates a significant difference ($p < 0.05$).

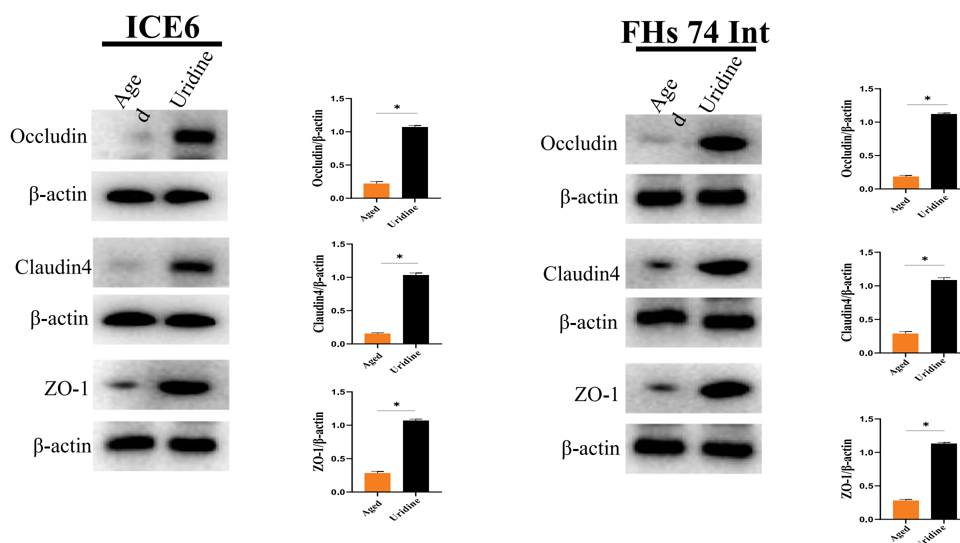

Figure 5. Occludin, claudin4 and ZO-1 expression were up-regulated in senescent IEC6 cells by uridine treatment. The total protein was extracted. Each sample (30 μg) was subjected to SDS-PAGE, and then transferred to the PVDF membrane. After three washes with TBST, the membranes were incubated with the primary antibody at 4°C overnight. The cell samples were washed and incubated with the secondary antibody for 1 h at RT. ($n = 5$). The asterisk indicates a significant difference ($p < 0.05$).

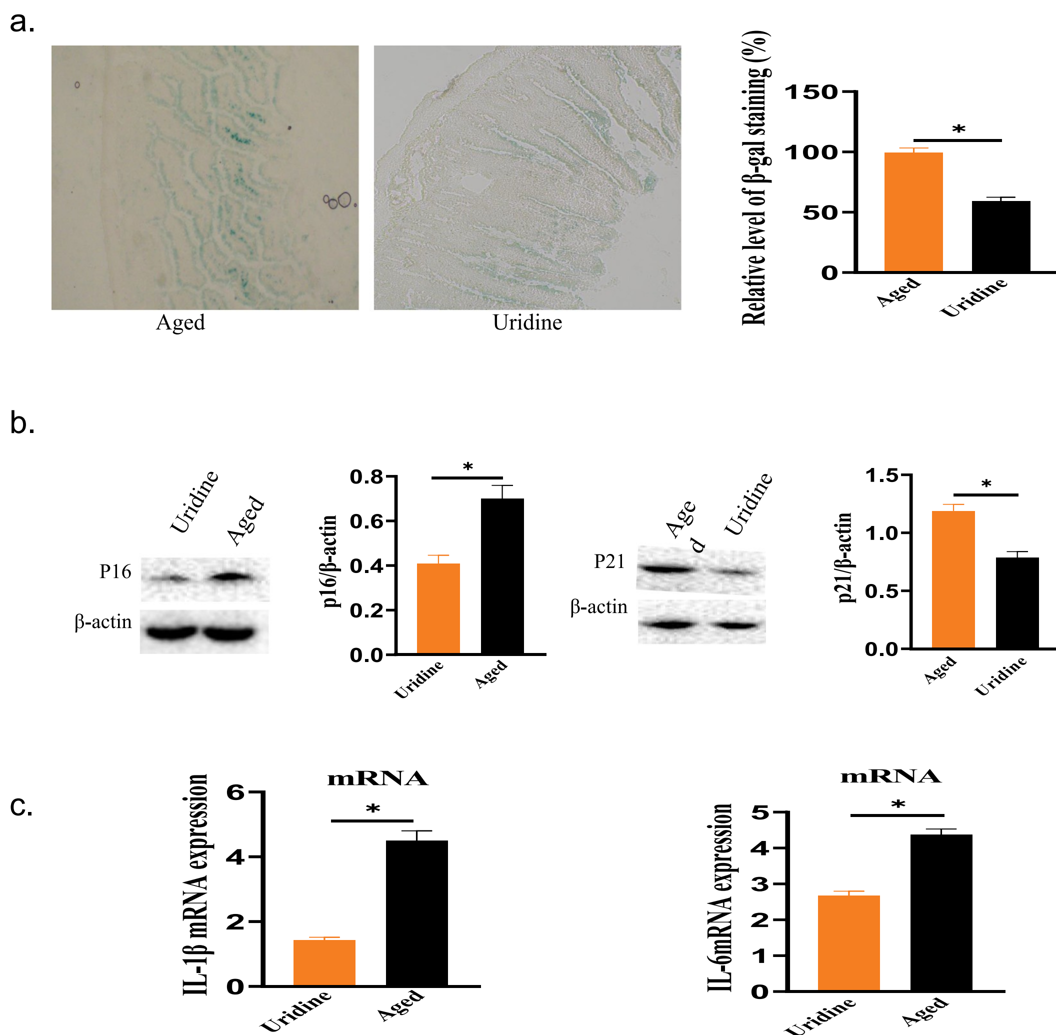

Figure 6. a. β-gal staining of intestine was reduced by uridine treatment. b. The expression levels of P21 and P16 were significantly reduced by uridine treatment. c. IL-6 and IL-1β were down-regulated by uridine treatment. d. Evaluation of the effect of uridine on the intestine by HE staining. Results were presented as means ± SD (n = 5). The asterisk indicates a significant difference ($p < 0.05$).

expression levels of tight junction-associated proteins were up-regulated by uridine treatment (Figure 5). These findings suggest the uridine could enhance the expression of intestinal barrier molecules.

Evaluation the effect of uridine on intestinal aging *in vivo*

We evaluated the effect of uridine on aged intestinal tract. The aged mice (21 month) were feed with uridine for one month (10 mg/kg). We found that uridine could significantly alleviate intestinal tract aging. As shown in Figure 6a, uridine significantly reduced the level of intestinal SA-β-gal staining compared to control group. Furthermore, the expression levels of P21 and P16 were also significantly reduced compared to control group (Figure 6b). In addition, the expression level of inflammatory factors was also reduced in uridine-fed mice (Figure 6c). We further analyzed the effect of uridine on the intestine by HE staining, and found that uridine could increase small intestinal mucosal thickness, villi length, but has no significant effect on crypt depth (Figure 6d). In addition, we evaluated the expression of P16 and P21 in intestinal tissue, as shown in supplementary Figure 1, P16 and P21 expression were significantly down-regulated in uridine treatment group. Furthermore, intestinal fibrosis (Fibrosis is closely

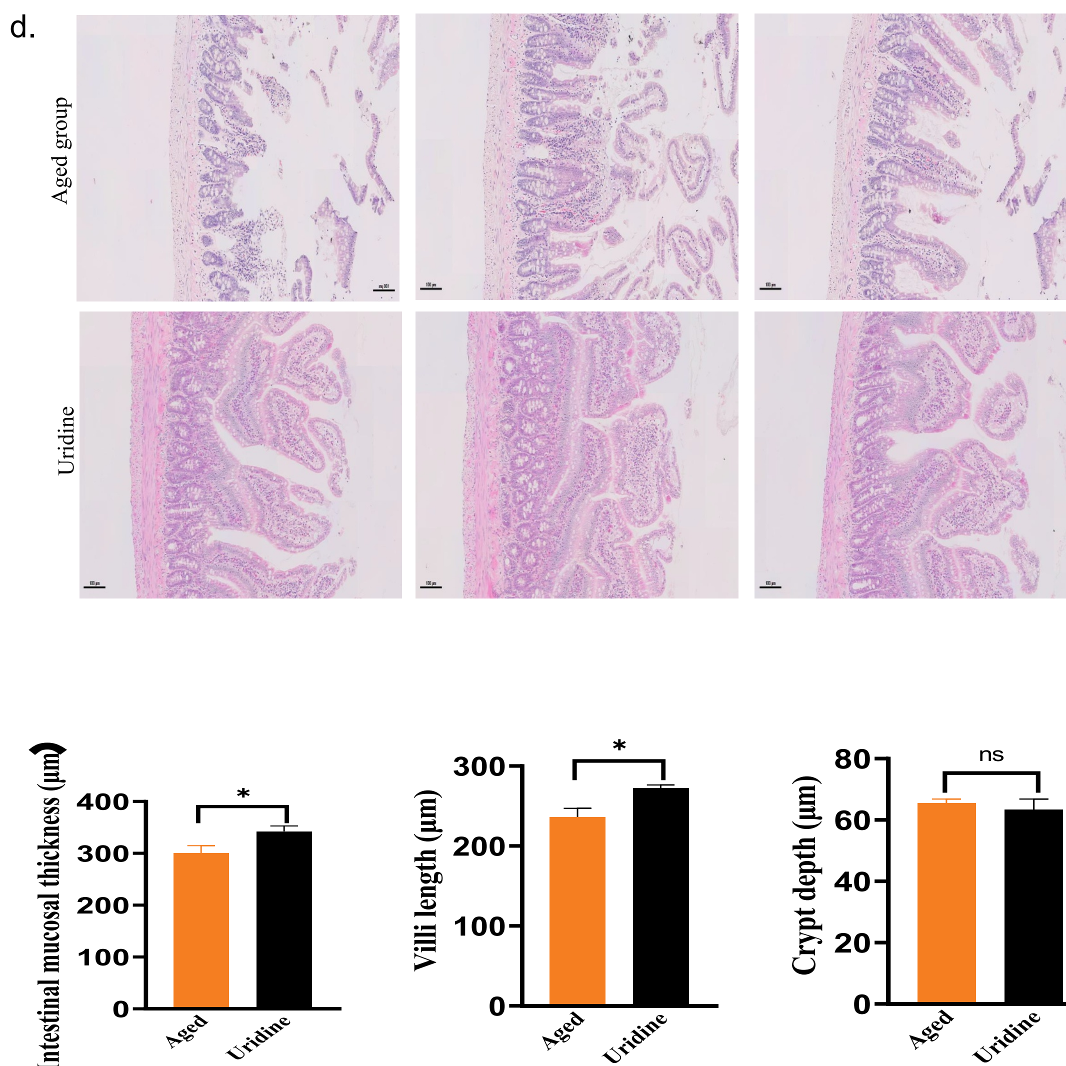

Figure 6. (Continued).

related to aging) was also significantly reduced compared to control group (supplementary Figure 2). Additionally, the proportion of CD68-positive cells was significantly down regulated by uridine treatment (supplementary Figure 3).

Discussion

At present, many countries in the world have begun to enter into the aging society [1]. With the growth of people's aging, the body's physiological function begins to decline, and the body's organizational structure will also change accordingly. Aging is a biological phenomenon that the body's physiological ability to adapt to the environment gradually decreases [2]. Aging can be divided into two types, physiological aging and pathological aging [14,15]. Aging is due to the imbalance of various homeostasis in the body over time. Until now, scientists have discovered some ways to relieve aging, such as removing aging cells.

It is well-known that intestinal tract is the main place for the digestion and absorption of nutrition [16], therefore the health of intestinal tract is an important premise to maintain body health. Aging could cause many changes in intestinal tissue structure. Intestinal aging can lead to mucosal defense defects and increase oxidative stress. Therefore, the prevention of intestinal aging is a hot issue nowadays. In the current study, we

explored the anti-aging effect of uridine, and the results showed that uridine could regulate the intestinal aging *in vivo* and *in vitro* experiments.

In the current study, we successfully established two intestinal cell senescence model by H_2O_2 treatment. We found that uridine could significantly reduce the IEC6 senescence. The expression level of Sa-β-gal was decreased significantly compared to control group. Further experiments showed that senescence markers (such as p21 and p16) were obviously down-regulated. These findings indicated that uridine has good anti-aging effect. In addition, the nuclear size and LAP2 intensity between control group and uridine-treated group were also analyzed, and the results also showed that uridine significantly inhibited the senescence of intestinal cells. Furthermore, cell proliferation assays showed that the cell proliferation ability in the uridine-treated group was significantly enhanced compared with the control group. In addition, a series of studies have shown that uridine has many positive effects. For example, uridine could regulate mitochondrial KATP channel [17]. Uridine treatment improves the learning and memory in a neonatal rat model [18].

As we all know, aging is closely related to inflammation [19]. Chronic inflammation can induce aging. Inflammation may be a key factor in the aging process. In the current study, we found that uridine could reduce the level of inflammation in the senescent cells. Further analysis of the underlying mechanism showed that uridine inhibits inflammation possibly via down-regulation of the activation of inflammation-related signaling pathway. These findings suggest that uridine could alleviate aging by regulating inflammation. It has been reported that uridine could inhibit pro-inflammatory responses induced by LPS stimulation [19]. In addition, Studies have shown that inflammation can induce cell senescence, which can release reactive oxygen species (ROS) and induce oxidative stress [20]. Oxidative stress further promotes cell senescence. In the current study, we found that uridine also could inhibit oxidative stress.

We further evaluated the anti-aging potential biological activity of uridine *in vivo*. The results showed that uridine could alleviate intestinal aging *in vivo*. Uridine significantly reduced the level of intestinal β-gal staining. The expression level of inflammatory factors was also reduced in uridine-fed mice. We further analyzed the effect of uridine on the intestine by HE staining, and found that uridine could increase small intestinal mucosal thickness, villi length, but has no significant effect on crypt depth. Taken together, these results indicate that uridine has anti-aging effect in vivo.

In the current study, we found that uridine exhibits the anti-aging effect *in vivo* and *in vitro*, which lays a foundation for further study of uridine's biological activities. As far as we know, this is the first study to find that uridine could relieve intestinal aging. This work suggests that uridine is expected to be used as a health food or drug to treat intestinal aging.

In conclusion, our current research shows that uridine shows a good anti-aging effect, which suggests that uridine is expected to be used as a health food or clinical drug to treat intestinal aging.

Acknowledgments

Thanks to Dr. Xu and Dr. Wang for their guidance on this work.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data Availability Statement

All data can be obtained from the corresponding author based on reasonable request.

References

[1] Dong-Heon K.employment policies in an aging society: review of the experiences of the oecd countries with population aging and their policy responses. East Asian Economic Review. 2017. 91:223–253

[2] Butler RN. Population aging and health. Br Med J. 1997;315(7115):1082–1084.

[3] Tian S, Wang J, Yu H, et al. Effects of galacto-oligosaccharides on growth and gut function of newborn suckling piglets. J Anim Sci Biotechnol. 2018;9(1):1–11.

[4] Nalle P, Pekka K. The role of stem cell niche in intestinal aging. Mech Ageing Dev. 2020;191:111330.

[5] Olea MT, Nagata T. A radioautographic study on RNA synthesis in aging mouse spleen after 3h-uridine labeling *in vitro*. Cell Mol Biol. 1992;38(4):399–405.

[6] Battaglia S, De Santis S, Rutigliano M, et al. Uridine and pyruvate protect T cells' proliferative capacity from mitochondrial toxic antibiotics: a clinical pilot study. Sci Rep. 2021;11(1):1–9.

[7] Petricevic M, Denko CW, Messineo L. Age-related changes in total DNA and RNA and incorporation of uridine and thymidine in rat liver, kidney and spleen. International Journal of Biochemistry. 1983;15(5):751–753

[8] Krylova IB, Kachaeva EV, Rodionova OM, et al. The cardioprotective effect of uridine and uridine-5′-monophosphate: the role of the mitochondrial ATP-dependent potassium channel. Exp Gerontol. 2006;41(7):697–703.

[9] Walker UA, Venhoff N. Uridine in the prevention and treatment of NRTI-related mitochondrial toxicity. Antivir Ther. 2005;10 Suppl 2(2):M117–23.

[10] Connolly GP, Duley JA. Uridine and its nucleotides: biological actions, therapeutic potentials. Trends Pharmacol Sci. 1999 May;20(5):218–225.

[11] Zheng WV, Li Y, Cheng X, et al. Uridine alleviates carbon tetrachloride-induced liver fibrosis by regulating the activity of liver-related cells. J Cell Mol Med. 2021;00:1–15.

[12] Jia Y, Jin Z, Chen S, et al. Uridine relieves MSCs and chondrocyte senescence in vitvo and exhibits the potential to treat osteoarthritis in vivo. Cell Cycle. 2022. DOI:10.1080/15384101.2021.2010170

[13] Nicoletti C. Age-associated changes of the intestinal epithelial barrier: local and systemic implications. Expert Rev Gastroenterol Hepatol. 2015;9(12):1467–1469.

[14] Jér R, Me D, Frédérique M. Physiological and pathological aging affects chromatin dynamics, structure and function at the nuclear edge. Front Genet. 2016;7(153):1–10.

[15] Jiang L, Shen Y, Guo D, et al. Author correction: epcam-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance. Nat Commun. 2020;11(1). 10.1038/s41467-020-17430-y

[16] Dandolini ST, Ravinder N, Hariom Y, et al. Senolytic combination of dasatinib and quercetin alleviates intestinal senescence and inflammation and modulates the gut microbiome in aged mice. J Gerontol A. 2021;76(11):1895–1905.

[17] Mironova GD, Khrenov MO, Talanov EY, et al. The role of mitochondrial katp channel in anti-inflammatory effects of uridine in endotoxemic mice. Arch Biochem Biophys. 2018;654:70–76.

[18] Cakir A, Kocoglu S, Serter K. Long-term cognitive effects of uridine treatment in a neonatal rat model of hypoxic-ischemic encephalopathy. Brain Res. 2017;1659:81–87.

[19] Kim DH, Lee B, Lee J, et al. Foxo6-mediated il-1β induces hepatic insulin resistance and age-related inflammation via the tf/par2 pathway in aging and diabetic mice. Redox Biol. 2019;24:101184.

[20] El Assar M, Angulo J, Rodríguez-Mañas L. Oxidative stress and vascular inflammation in aging. Free Radic Biol Med. 2013;65:380–401.