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SHORT COMMUNICATION

Effect of tranexamic acid in improving the lifespan of naturally aging mice

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Abstract
An effective method to improve lifespan is not known. Therefore, in this study, we examined the lifespan-extending effect of tranexamic acid in normal mice. We bred hairless mice without exposure to ultraviolet radiation and psychical stress until they died naturally. During the study period, the mice were orally administered tranexamic acid (12 mg/kg/day) three times weekly. An increase in the lifespan of mice was observed by tranexamic acid administration. Furthermore, age-related diseases of the skin were ameliorated by tranexamic acid administration. Moreover, the blood level of tumor necrosis factor-α, interleukin-6, reactive oxygen species (ROS), and matrix metalloproteinase (MMP)-9 was decreased by tranexamic acid administration. These results indicate that tranexamic acid suppresses the secretion of inflammatory cytokines, MMP-9, and ROS induced by natural aging, ameliorating age-related diseases, and, consequently, extending the lifespan.

Keywords Interleukin-6 · Matrix metalloproteinase-9 · Natural aging · Reactive oxygen species · Tranexamic acid · Tumor necrosis factor-α

Introduction

Recently, the relationship between aging and inflammation has been elucidated. There are two types of inflammation. Acute inflammation is induced by vulnus and bacterial or viral infection, leading to rubor, tumorigenesis, thermacogenesis, and pain (Mababe 2017). Chronic inflammation does not manifest symptoms like acute inflammation; under chronic inflammation, the inflammation smolders (Shaw et al. 2013). Furthermore, chronic inflammation is associated with aging, articular rheumatism, arteriosclerosis, diabetes, and Alzheimer's disease (Franceschi and Campisi 2014). In general, chronic inflammation occurs by natural aging of tissues. For example, a decrease in immunity (Arnardottir et al. 2014), continuous secretion of inflammation-aging signal, called senescence-associated secretory phenotype (Ohtani et al. 2007), systemic metabolism, and endocrinology alteration accompanying aging also promote inflammation (Abu-Taha et al. 2009). Thus, various factors accompanying aging induce chronic inflammation. The occurrence of diseases increases rapidly at the age of 40–50 in human, the chronic inflammation that does not manifest symptoms is a concern. Furthermore, the negative spiral continues to increase with chronic inflammation and aging.

Tranexamic acid (trans-4-aminomethylcyclohexanecarboxylic acid) is known as a safe and traditional medicine with anti-plasmin function. Plasmin causes disengagement and disassembly of arachidonic acid, increasing the level of prostaglandin, which is a metabolite of arachidonic acid (Okunishi et al. 2011). Prostaglandins are associated not only with the induction of acute inflammation, but also with the secretion of cytokines related to chronic inflammation (Okunishi et al. 2011). Owing to its anti-plasmin function, tranexamic acid inhibits arachidonic acid disengagement and prostaglandin production, exhibiting an anti-inflammatory effect (Isseroff and Rifkin 1983). Previously, we reported the skin photo-aging-ameliorating effect of tranexamic acid (Hiramoto et al. 2018). However, the lifespan-extending effect of tranexamic acid against natural aging has not been investigated.

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In this study, we examined the lifespan-extending effect of tranexamic acid in naturally aging mice. We continuously administered tranexamic acid to mice until death and compared the lifespan of treated and non-treated mice. In addition, we investigated the relationship between lifespan and inflammation.

Materials and methods

Animal experiments

Eight-week-old specific-pathogen-free (SPF) male hairless mice (SLC, Hamamatsu, Shizuoka, Japan) were used. The mice were maintained individually in cages in an air-conditioned room at 23 °C ± 1 °C under SPF conditions with a 12-h light/12-h dark cycle. The light source used was a fluorescent lamp with ultraviolet ray filter (FLR110H.EX-D/M/36 WAN; Everise Inc., Maebashi, Gunma, Japan). We bred the mice under stress-free condition as much as possible. There were ten mice per group. The groups were as follows: control, solvent administration, and tranexamic acid administration. Each mouse was observed until it died naturally (lifespan). Blood samples were collected 30 months after the start of the experiments. We applied for this study to the "Suzuka University of Medical Science Animal Experiment Ethics Committee" and it was approved on September 25, 2014. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Suzuka University of Medical Science (approval number: 34). All surgeries were performed with the mice under pentobarbital anesthesia, and efforts were made to minimize animal suffering.

Tranexamic acid treatment

Approximately 12 mg/kg of tranexamic acid (Daiichi Sankyo Healthcare Co., Ltd., Tokyo, Japan) in distilled water was orally administered to the mice three times a week until the mice died. The solvent-administered group mice were administered distilled water. This value of 12 mg/kg/day was converted from that of human (750 mg/60 kg) to the value used for mouse (Hiramoto et al. 2019).

Measurement of plasma interleukin-6, tumor necrosis factor-α, reactive oxygen species, and matrix metalloproteinase-9

Blood samples were collected from the heart of the test mice 30 months after the start of the experiment. The plasma levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, and matrix metalloproteinase (MMP)-9 were determined using commercial enzyme-linked immunosorbent assay kits (IL-6: BioLegend, San Diego, CA, USA; TNF-α and MMP-9: R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturers' instructions. The plasma ROS level was determined using an OxiSelect^{TM} STA-347 in vivo ROS/RNS assay kit (Cell Biolabs, Inc., San Diego, CA, USA) in accordance with the manufacturer's instructions.

Statistical analyses

All data are presented as means ± standard deviation. The results were analyzed using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA). Differences between groups were evaluated using the one-way analysis of variance, followed by Tukey's post hoc test in SPSS version 20 software (SPSS Inc., Chicago, IL, USA). The results were considered significant at $p < 0.05$. Survival data were analyzed using Kaplan–Meier and Log-rank tests for survival distribution. Mann–Whitney test was used for statistical comparison of survival data ($p < 0.01$).

Results and discussion

The lifespan of the tranexamic acid-administered mice was extended compared with that of other two groups (Fig. 1). Furthermore, there was no difference between the control and solvent-administered groups in terms of lifespan. In the control and solvent-administered 30-month-old mice, tumors, tylosis, and vulnus were observed on the skin. However, in the tranexamic acid-administered mice, the deterioration of the skin condition was not observed (Fig. 2). The plasma levels of IL-6, TNF-α, MMP-9, and ROS were lower in the tranexamic acid-administered mice than those in the control and solvent-administered mice (Fig. 3). Furthermore, there was no difference between the control and solvent-administered mice.

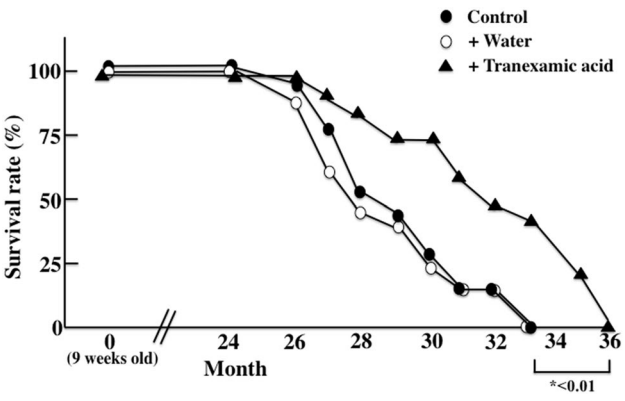

Fig. 1 Effect of tranexamic acid on the lifespan of naturally aging mice. The lifespan differed significantly between naturally aging mice treated with or without tranexamic acid. Log-rank test, $*p < 0.01$

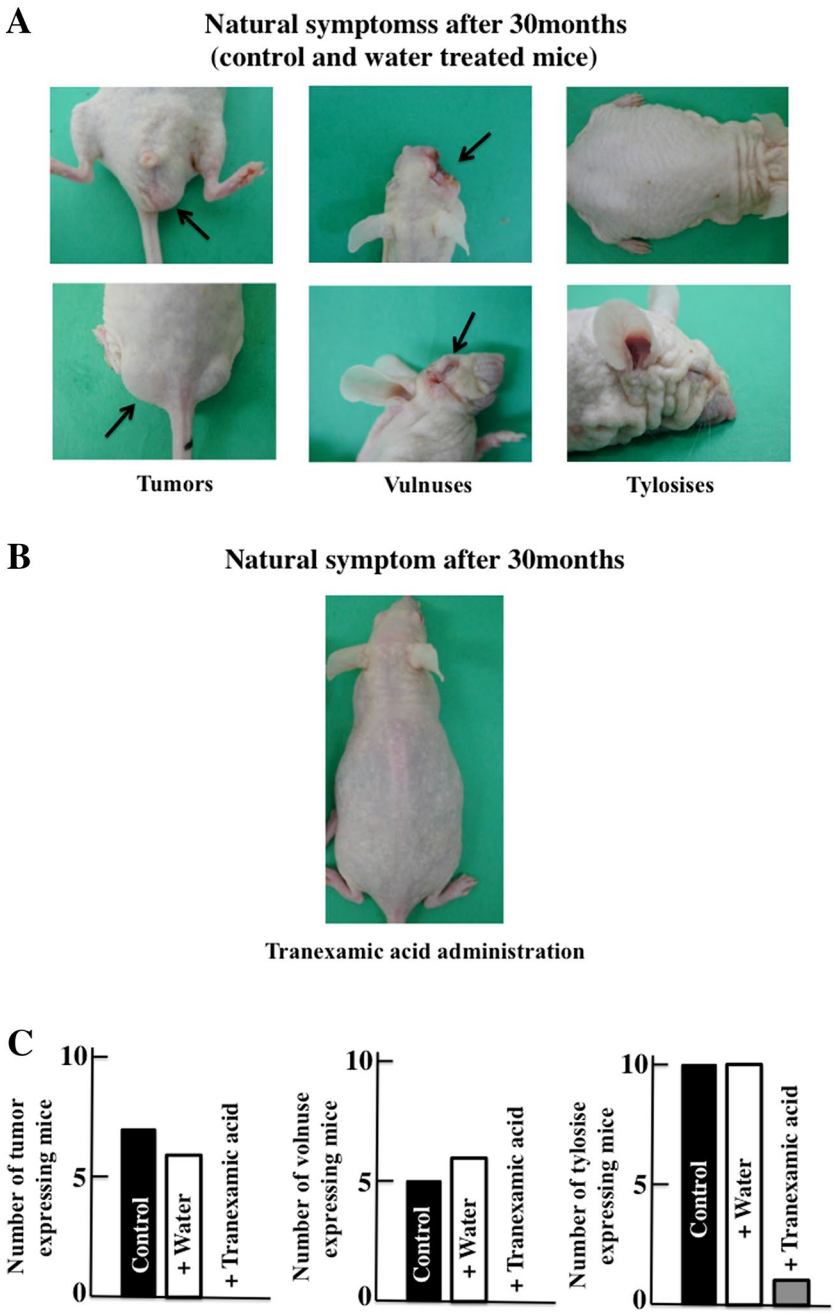

Fig. 2 Skin condition of 30-month-old mice

In this study, the lifespan of tranexamic acid-administered mice was extended significantly. In the control and solvent-administered mice, tylosis, tumors, and injury were observed on the skin. However, most of these symptoms were not observed in tranexamic acid-administered mice. In the 30-month-old mice, the blood levels of IL-6, TNF-α, MMP-9, and ROS decreased compared with those in the control and solvent-administered mice.

In general, inflammatory cytokines are released from various parts of a body to the pathological site, with the accumulation of immunocytes. The accumulated immunocytes attack foreign substances using active oxygen. Collagen then repairs tissue damage, and subsequently, capillary vessels and cells are reproduced (Zuk et al. 2002). However, under abnormal immunological response, a chronic inflammation is induced. In chronic inflammation, immunocytes continue to secrete ROS (Shimizu and Marusawa 2011). The ROS attack normal cells and result in the formation of tumors.

It has been reported that aging of cells induces systemic aging by aging-associated diseases. Inflammatory cytokine secretion increases in this process. Especially, the levels of IL-6 and TNF-α increase considerably. Furthermore, TNF-α increases the levels of nuclear factor-kappa B (NF-κB) and IL-6, increasing the level of signal transducer and

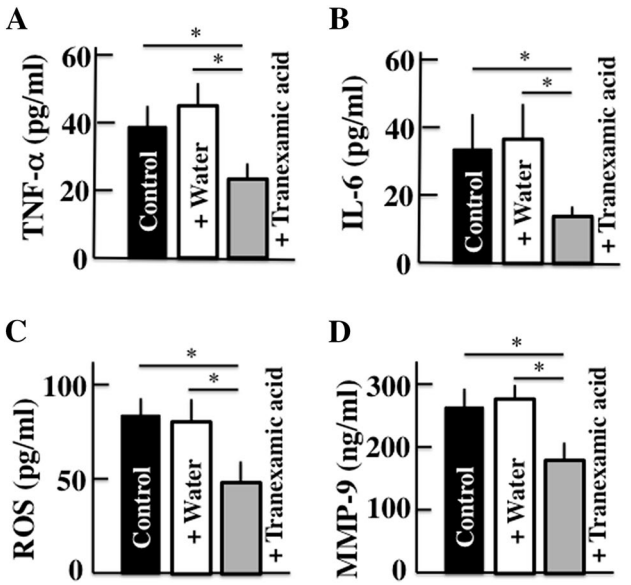

Fig. 3 Effect of tranexamic acid on the plasma levels of TNF-α (**a**), IL-6 (**b**), ROS (**c**), and MMP-9 (**d**) in 30-month-old mice. The values are expressed as the mean ± SD of ten animals. *$p < 0.05$

activator of transcription 3 (STAT3). NF-κB and STAT3 in turn increase the IL-6 level, amplifying the inflammation circuit (Ma et al. 2017; Neurath and Finotto 2011; Yoshizaki 2008).

Thus, in chronic inflammation/aging, ROS, IL-6, and TNF-α play important roles. Contrarily, inhibition of secretion of these cytokines and ROS was observed with the administration of tranexamic acid (Fig. 3), indicating a decrease in the inflammation circuit. Therefore, tranexamic acid was considered to increase the lifespan by inhibiting the levels of ROS, IL-6, and TNF-α, which are involved in chronic inflammation, aging, and aging-associated diseases.

Tranexamic acid inhibits plasmin production (Ng et al. 2015). Upon perceiving the signal of inflammation, plasmin disassembles kininogen, which is component of kinin, produces kinin, and induces swelling and pain. Tranexamic acid inhibits plasmin function, decreases kinin production, and suppresses inflammation (Yamasaki et al. 1967). In addition, plasmin processes the cytokines through MMP-9 and activates inflammatory reaction (Tsuji et al. 2005). There was a decrease in the production of MMP-9 with the administration of tranexamic acid (Fig. 3). From these functions, it was thought that tranexamic acid suppresses inflammation by inhibiting the production of IL-6, ROS, and TNF-α, at the onset of aging-associated diseases by chronic inflammation, and, consequently, increasing the lifespan. However, the relationship between plasmin inhibition by tranexamic acid and chronic inflammation is not clear, and further examination is necessary in this regard.

Conclusion

Tranexamic acid extends the lifespan of naturally aging mice, by inhibiting the synthesis of plasmin and decreasing the blood level of ROS, IL-6, MMP-9, and TNF-α. Thus, tranexamic acid inhibits the onset of aging-associated diseases. Overall, aging-associated diseases and aging can be ameliorated by the use of tranexamic acid. Further studies should be conducted to elucidate the relationship between plasmin inhibition and inflammatory cytokine secretion (chronic inflammation), which can help to develop novel therapeutic methods utilizing tranexamic acid.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest in association with this study.

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