# Chapter 25
# Simulative Evaluation of Taurine Against Alopecia Caused by Stress in *Caenorhabditis elegans*

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**Abstract** Hair loss or alopecia has been portrayed as a modern malady which is aggravated by stressful conditions. Major cases of alopecia were found among individuals of 40s–50s, nowadays, even among the 20s–30s. This study characterized taurine's potential against alopecia caused by chemical stress agents based on the comparison with other commercially available anti-alopecia agents using *Caenorhabditis elegans*. The criteria used are their effects on the expression of stress markers and measurements of vital signs: lifespan comparison, progeny number, and mobility. *C. elegans* showed the typical stress symptoms under treatment with tunicamycin, endoplasmic reticulum stress agent. Hsp-70 protein expression increased, while worm's lifespan and per capita progeny number significantly decreased along with an unusually retarded movement. A positive response was shown when worms were treated with taurine along with astressin-B and finasteride. Between the treatments, finasteride showed better outcomes in terms of stress-reducing effects. Taurine helped worms recover more effectively from adverse influence of stress. In conclusion, there is strong evidence that taurine has a great potential as anti-alopecia effect especially against the one caused by the chemical stress. The present study implies that taurine might strongly work against hair loss when used in combination with other commercially available anti-alopecia agents.

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## Abbreviations

| | |
|---|---|
| Tau | Taurine |
| Tun | Tunicamycin |
| NGM | Nematode growth medium |
| ER | Endoplasmic reticulum |
| RR | Rescue rate |

## 25.1 Introduction

Hair is a characteristic feature of mammals and has significant biological usages. Hairs grow out of the epidermis to develop a shield against potential damages from ultraviolet light, heat, and external harmful debris. Humans have shown a trend to eliminate hairs during the course of evolution from their ancestors. In hair, living part exists only at the root part; however, its functional integrity depends on the whole structure. Thus, hair can be considered as continuous functional structure from root to end and the strength of hair root greatly affects the entirety of hair.

Alopecia refers to the general sense of hair loss from the head and baldness is usually the typical outcome of alopecia. Earlier alopecia exerts a significant sense of frustration especially to younger generations. The cause of alopecia is not well understood to date; however, modern stressful living conditions appear to coincide with alopecia for humans. There are three major categories in terms of cause and development of alopecia (Fig. 25.1). Alopecia results from extended cellular stress in hair root cells along with individual genetic background. Combined with individual genetic background, alopecia can occur at early stage of life and intensifies with stressful circumstances. The chance for alopecia increases with age and males are more susceptible to alopecia than females (Table 25.1, compiled from Dior 2011).

Extreme physical stress is believed to worsen the health of hair (Novak and Meyer 2009). Among adolescents, alopecia is triggered by unusual hair care maintenance, such as twisting hair treatments, bleaching, and behavioral pulling of hair. Recent studies indicate that alopecia is also caused by a type of organelle stress such as endoplasmic reticulum stress (Gupta et al. 2009; Sarkar et al. 2011). According to the theory, various stresses affect the cellular conditions within stem cells in hair follicles and the eventual apoptosis depreciates the stem cells of hair follicles. Under circumstances, anti-ER stress compounds may reduce the occurrence of alopecia and even avoid the symptom when its application is implemented beforehand at high-risk groups.

A number of medicines are available to treat the symptoms related to alopecia. Majority of the medicine has been developed to strengthen the integrity of hair at its

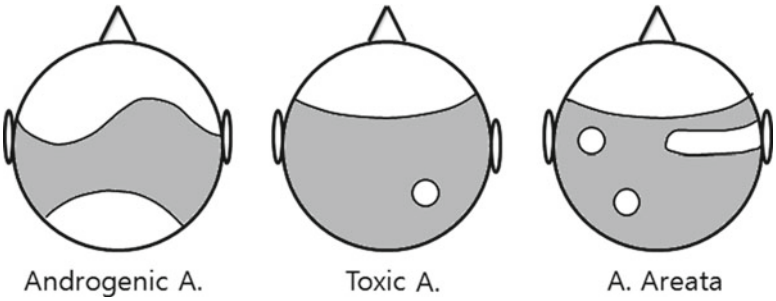


**Fig. 25.1** Top-down view of different alopecias. Alopecia (A.) varies in shape and pattern of development. Androgenic A. and A. areata are affected mostly by individual genetic backgrounds. The areas of hair loss, caused by androgenic A. and A. areata, expand with age. Toxic A. is affected by environmental stresses and can be cured with relevant treatments

**Table 25.1** Alopecia statistics

| Sex | Characterization | (%) |
|---|---|---|
| Male | –Androgenic in all baldness | 95 |
| | –Showing signs of balding by 50s | 67 |
| | –With male pattern baldness by 50s | 50 |
| | –Balding by 30s | 25 |
| Female | –Suffering hair loss by 50s | 25 |

root. Recently, astressin-B has been approved as anti-alopecia medicine by the FDA of the United States as a nonselective antagonist to corticotropin-releasing hormone. It prompted copious hair growth in mice which was subjected to an alopecia treatment (Wang et al. 2011). Finasteride is used to treat alopecia especially in the form of male pattern baldness as well as benign prostatic hyperplasia. Finasteride inhibits the conversion of testosterone to dihydrotestosterone (Evers et al. 2010).

Hair growth and regeneration test model is available in vitro and in vivo. Mice are frequently used as model systems in evaluating anti-alopecia candidate agents (Porter 2003). This study, however, utilizes *Caenorhabditis elegans* as the model system considering they correctly represent cellular stress leading to ER stress at the individual level (Link et al. 2001; Silverman et al. 2009). Under treatment of candidate agents including taurine, their response to the ER stress will be very comprehensive and less laborious. In this study, the two commercially available anti-alopecia agents were evaluated in terms of ER stress level along with taurine: The extent of anti-ER stress effect was evaluated according to their effect on the stress protein expression and, functionally, on the basis of life length, mobility, and offspring numbers which constitute very useful indicators for physiological stress (Estes et al. 2005; Ayyadevara et al. 2007; Boyd et al. 2007; Davies and Hart 2008).

## 25.2 Methods

### *25.2.1 Synchronous Culture Conditions and Induction of ER Stress*

Wild type of *C. elegans* N2 was cultured at 25°C on the nematode growth medium (NGM) according to the standard method (Stiernagle 2006; Szewczyk et al. 2003). To induce ER stress, worms were grown for 3 h on media containing tunicamycin at 10 μg/ml. They were further incubated with various concentrations of anti-alopecia agents. The anti-alopecia agents used in this experiment were astressin-B, finasteride, and taurine which were all purchased from Sigma (St. Louis, USA). The anti-alopecia agents were delivered into the media as the final concentration of 10 or 100 μg/ml. The stress conditions were assessed by examining the stress protein marker expression. The expression of hsp-70 was detected according to the standard immunoblotting procedures. The antisera against hsp-70 were purchased from Santa Cruz Biotechnology (Santa Cruz, USA) and diluted as 1,000 times for immunoblotting in the present experiments.

### *25.2.2 Measurement of Worm's Vital Signs: Lifespan Extension, Progeny Number, and Mobility Restoration*

The effect on life length extension was estimated using the method of Hyun et al. (2008). As a brief description, worms were initially destroyed except the eggs by bleaching and ten eggs were incubated on NGM supplemented with OP50 at 25°C until worms reached the young adult stage. Fifty worms were then transferred onto plates containing 10 μg/ml of tunicamycin, where they were incubated for 3 h. They were subsequently transferred to media containing 10 or 100 μg/ml of astressin-B, finasteride, or taurine. Live worms were counted daily. Worms were excluded from counting if they failed to react to a stimulus with a platinum wire. The lifespan extension effect was calculated as in a rate of rescuing worms from the chemical stress. The rescue rate (RR, %) was calculated according to the following formula: RR(%)=(A−B)/(C−B) X 100(%), where A refers to offspring number with each drug treatment after Tun, B to offspring number with Tun-only treatment, and C to offspring number with drug-free treatment.

In order to evaluate the effect on offspring number, the number of eggs was counted daily and standardized per number of adult after the anti-alopecia treatment under the ER stress conditions. The adults were selected for visual consistency and removed to a fresh plate with 10 μg/ml of tunicamycin. After subsequent 3 h incubation on the Tun media, they were further treated with anti-alopecia agents at 10 or 100 μg/ml. The number of fertilized eggs and larvae was counted and standardized as a rate of increase on 2, 3, and 4 days after the beginning of the culture.

The mobility of worm was assessed by monitoring the total moving length of the worms in the presence of anti-alopecia agents. As in the life duration experiment above, worms were treated with 10 μg/ml of tunicamycin and relocated to the anti-alopecia media with their usual content at 10 or 100 μg/ml. After the repositioning into the anti-alopecia media, the extent of movement was determined according to the turbidity of the NGM media. Their turbidity was visually compared against anti-alopecia-free.

## 25.3 Results

In order to characterize taurine's potential as anti-alopecia agent against the hair loss caused by stress, this study focused on whether it reduces the level of stress caused by tunicamycin in *C. elegans* along with other leading anti-alopecia agents: astressin-B and finasteride. Two categories of assay were performed. Firstly, the expression of hsp-70, ER stress marker, was measured and the overall level of hsp-70 increased after the tunicamycin treatment but significantly diminished under treatment with taurine. As a second line of approach, measurements were made focusing on the level of vital signs: survivorship, offspring number, and mobility of the worms. In terms of these functional markers, taurine and the two anti-alopecia agents caused a reduction in ER stress by restoring the vital signs toward those of the no stress controls.

### *25.3.1 Reduction of Hsp-70 Under Tau Treatment After Tun*

The expression of hsp-70 significantly increased compared to no stress control when the worms were treated with tunicamycin. In the presence of taurine, however, the level of hsp-70 expression was significantly affected. The extent of hsp-70 expression showed a dose-dependent manner, along with the amount of the tunicamycin added (Fig. 25.2). When incubated with astressin-B or finasteride, the worms failed to show any significant variation in terms of hsp-70 protein expression (data not shown). The data imply that taurine may lower the level of ER stress caused by tunicamycin, since the reduced expression of hsp-70 represents the decreased level of ER stress in the worms.

### *25.3.2 Higher Rescue Rate Under Taurine Treatment*

The treatment with tunicamycin curtailed the lifespan of worms. Beginning two days after the start of the culture, tunicamycin was shown to adversely affect the growth of *C. elegans* according to the significant reduction of worms in number

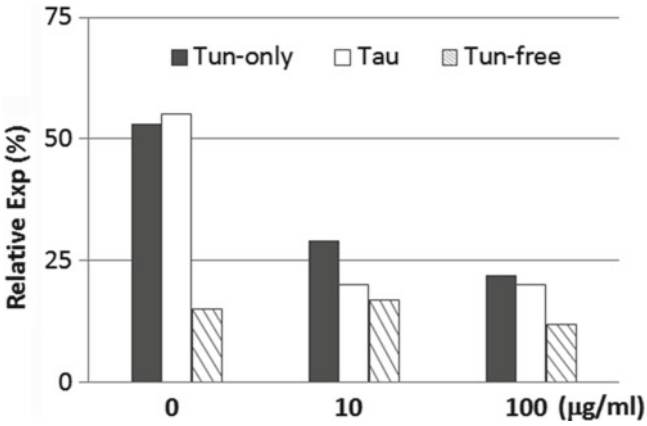


**Fig. 25.2** Effect of taurine on hsp-70 expression in ER-stressed *C. elegans*. The expression of hsp-70 was compared at the three concentrations of taurine. Tun-treated worms showed a higher expression of hsp-70 compared to no Tun (or no stress) treatment. Along the increase of taurine concentration, however, the level of hsp-70 expression decreased significantly

when they were treated singularly with tunicamycin. A significant worm fraction perished within 20 days after the start of the treatment. When the worms were treated with the three candidate agents, their rescue rate increased in comparison to Tun-only treatment (Fig. 25.3). Among the three candidate chemicals, taurine notably enhanced the rescue rate. Both astressin-B and finasteride exerted positive effects on the rescue rate although taurine surpassed the two anti-alopecia agents considerably. These data indicate that the agents against alopecia may help the worms recover from the chemical stress by Tun. Also, the enhanced rescue rate strongly implies that the agents may help the extension of worm's lifespan which has been significantly shortened under chemical stress by Tun.

### *25.3.3 Progeny Number Recovered Under Treatments with Anti-Alopecia Agent*

Each offspring number was recorded daily up to 4 days following the application of the three candidate molecules to the tunicamycin-treated worms. When worms were treated with Tun, they produced much less offspring compared to the Tun-free treatment. Nevertheless, the worms appeared to recover from the effect of Tun when they were transferred to the media containing the respective candidate chemicals (Fig. 25.4). Both finasteride and astressin-B show a positive effect on the number of progeny. Compared to the two chemicals, taurine showed a more positive effect on the ER-stressed worms. Throughout the 2–4 day count, taurine-treated worms produce at least 20% more offspring than Tun-only-treated worms on the per capita basis. These results indicate that the three anti-alopecia agents help the worms to

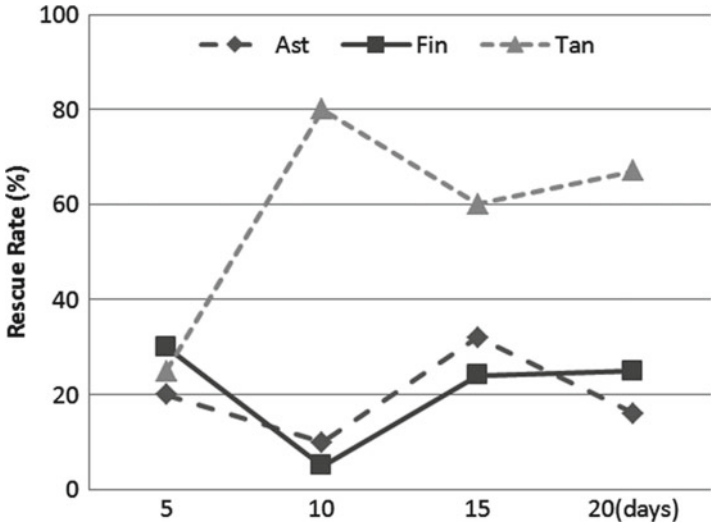


**Fig. 25.3** Effect of anti-alopecia agents on lifespan extension. The effect of anti-alopecia agents on lifespan was calculated as rescue rate according to the description in Sect. 25.2. Taurine significantly prolonged worm's lifespan; however, astressin-B and finasteride slightly helped worms to recover from the stress compared to taurine

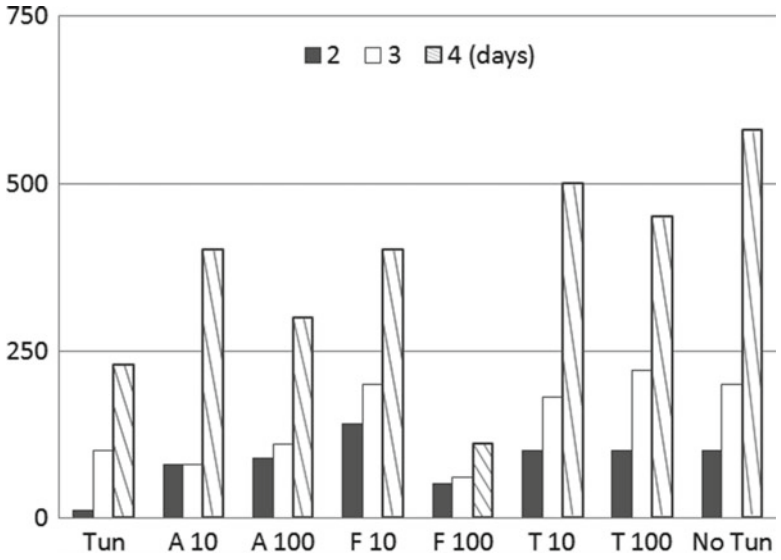


**Fig. 25.4** Progeny number under treatments with anti-alopecia agents. Progeny numbers were scored daily after worms were treated with astressin-B (A), finasteride (F), and taurine (T) at 10 or 100 μg/ml for 2–4 days. Under taurine treatment, the offspring number was consistently greater than those of A and F

**Table 25.2** Comparison of mobility after drug treatment

| | Conc.(μg/ml) | | |
|---|---|---|---|
| Drug | 0 | 10 | 100 |
| Astressin-B | + | ++ | + |
| Finasteride | + | + | ++ |
| Tau | + | ++ | +++ |
| Tun-only | + | + | + |

recover from the ER stress at the level of organism. The increased number of offspring may originate from the anti-ER stress effect of the three candidate molecules.

### *25.3.4 Restoration of Mobility Among Worms Treated with Anti-Alopecia Agents*

To evaluate the effect of anti-alopecia agents on the mobility of the stressed worms, their moving distances were figured out according to the turbidity which was caused by the worm's path. The turbidity was visually compared at three different concentrations of the candidate chemicals. When treated with Tun, worms showed a considerable decline in mobility. The reduced movement of worms, however, apparently recovered to reach the level of ER stress-free control when worms were treated with taurine or two other alopecia agents following taurine application. Table 25.2 summarizes the mobility of worms cultured at two different concentrations of the three drugs, as expressed in the turbidity of the media. Along with astressin-B and finasteride, taurine facilitates the worms to recover from the stress. This result strongly implies that taurine helped the worms recuperate from the adverse influence of ER stress on their mobility.

## 25.4 Discussion

A considerable percentage of adults and youths suffer severe emotional stress from alopecia. Alopecia occurs in adolescents even at the age of 14. Astressin-B and finasteride cannot be applied to the adolescents less than 18 years old and hair implant is not a feasible alternative at this age, and development of safe anti-alopecia agents is very important under the circumstances. The present study utilized *C. elegans* to develop an assay system for anti-alopecia drugs which are especially effective against stresses.

*C. elegans* displayed usual ER stress signs under tunicamycin with treatment such as augmented expression of hsp-70, heat shock proteins. The tunicamycin

treatment caused many physiological stress symptoms: decreased survivorship, stunted movement, and reduced number of offsprings. When the worms were treated with the commercial anti-alopecia agents and taurine, however, they showed positive responses against ER stress conditions. Although there is a variation among the three agents, they showed positive rescue rates and increased mobility and progeny number.

Although astressin-B and finasteride are two prominent drugs to treat alopecia, the data in this study suggest that taurine may work better than the two anti-alopecia agents against hair loss caused particularly by stresses. These results strongly implicate that taurine might alleviate the chemical stress to help hair root cells to sustain their integrity.

Future study may be meaningful to characterize whether a synergistic effect may exist among the agents used in this study. Also, a new study should employ a mouse hair loss system to verify the results achieved from the present *C. elegans* model study.

## 25.5 Conclusion

Considering hair loss causes adolescent to suffer devastating emotional stress, the significance of safe, effective anti-alopecia agent cannot be overstated. Development of screening systems for anti-alopecia agents is very important. This study used *C. elegans* to evaluate astressin-B, finasteride, and taurine in terms of anti-stress potential. All of them helped with restoring the affected vital signs in *C. elegans* which was subjected to ER stress. Among the three agents, taurine exerts better results and may be used as an anti-alopecia agent especially against the one caused by stress.

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