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Assessment of the protective effect of D-methionine on hearing in acoustic trauma

Dissertation for the degree of Doctor of Medical Sciences

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SUMMARY

Noise-induced hearing loss (NIHL) is one of the most common occupational diseases, and noise is one of the most serious environmental pollution associated with the industry in developing countries. The reports published by the WHO show that over 466 million people in the world have disabling hearing loss, of which more than 40% of people are affected by moderate and severe hearing loss significantly reducing their quality of life. In spite of many actions leading to the reduction of noise level, this is a factor that cannot be eliminated due to the presence in every area of human activity, in particular in the work environment.

Oxidative stress is considered the main mechanism leading to the development of NIHL. It has been observed that even within 1-2 hours after exposure to excessive sound levels there is an increased production of reactive oxygen species (ROS) in the inner ear and this level lasts up to 10 days. It is also significant that the elevated levels of these molecules were found in the auditory cells before any damage was observed.

A better understanding of the mechanisms of noise impact on the hearing system enables taking more and more effective steps in the therapy of hearing loss caused by this factor. Due to the significant role of oxidative stress in the pathogenesis of NIHL, most attention is paid to compounds increasing endogenous antioxidant defense - antioxidants. Among the potential therapeutic compounds in this group is methionine, a natural amino acid found in the human diet. Animal studies have shown that the D-isomer of methionine (D-Met) administered orally, by intraperitoneal injection or directly on the round window causes a reduction in permanent threshold shifts in the case of exposure to ototoxic substances (cisplatin, gentamicin) and noise. The promising results of work on animal models led to the start of clinical trials in 2013 of D-Met supplementation in people exposed to noise. Insufficient knowledge on the molecular mechanisms of D-Met action, the lack of works on impacts of D-Met for the expression of oxidative stress genes in acoustic trauma in particular, was the premise for further research into D-Met in the doctoral dissertation.

The aim of the study

The main objective of the study was to assess the mechanisms of protective effect of D-methionine (D-Met) on the hearing of mice in acoustic trauma.

The detailed objectives included:

1. Comparison of hearing threshold shifts after exposure to noise in mice treated and non-treated with D-Met in relation to control animals, which were not exposed to noise.
2. Comparison of activity of selected oxidative stress markers after exposure to noise in mice treated and non-treated with D-Met in relation to control animals, which were not exposed to noise.
3. Comparison of gene expression of selected oxidative stress markers after exposure to noise in mice treated and non-treated with D-Met in relation to control animals, which were not exposed to noise.
4. Determination of the effective dose of D-Met protecting against acoustic trauma in mice.

Material and methods

In this study six week old male C57BL/6 mice with a recessive gene mutation on the chromosome 10 locus *ahl* 1 (age-related hearing loss locus 1), characterized by an accelerated hearing loss associated with exposure to noise, were used. The animals were randomly divided into five groups:

I – **Control group** (no exposure to noise, no administration of D-met)

II – **Noise group** (exposed to noise only)

III – **D-Met 100 group** (exposed to noise and treated with D-met at 100 mg/kg)

IV – **D-Met 200 group** (exposed to noise and treated with D-met at 200 mg/kg)

V – **D-Met 400 group** (exposed to noise and treated with D-met at 400 mg/kg)

The tests were carried out at 3 time points: 1, 7 and 14 days after exposure to noise.

Hearing tests were performed on a total of 289 animals, of which the material for biochemical assay was taken from 200 mice, and for genetic tests - from 53 mice.

Animals were exposed to 4 kHz octave band noise at the equivalent continuous sound pressure level of 110 dB (SPL) in a ventilated sound exposure chamber for 8 hours.

D-Met was dissolved in normal saline and delivered by intraperitoneal injection. D-Met at 100, 200 or 400 mg/kg per dose was administered 1 h before and 1 h after noise exposure. Additional doses were administered twice a day at the same time intervals on days 1, 2 and 3 following noise exposure.

The evaluation of hearing threshold shifts was carried out using the auditory brainstem response (ABR). In order to assess damage, hearing threshold values obtained before and after noise exposure were compared, thus obtaining values of shifts in noise thresholds for each subject examined.

Due to the small amount of cochlear tissue, for the evaluation of oxidative processes, measurements of the level of activity of oxidative stress markers - superoxide dismutase and catalase, were used. The dissected cochleae from the temporal bone of mice were homogenized in PBS buffer followed by determination of total superoxide dismutase activity using the SOD Assay Kit (Cayman Chemical Company) and catalase - using the Catalase Assay Kit (Cayman Chemical Company)

The evaluation of gene expression encoding selected oxidative stress markers (*Sod1, Sod2, Cat*) in inner ear tissues was performed by polymerase chain reaction with real-time PCR analysis, preceded by reverse transcription (RT-PCR). As a template for cDNA synthesis, RNA isolated from the mouse cochlea by modified Chomczynski method was used. To determine the expression of genes, the samples were normalized to the reference gene – *Hprt1.*

Results

Hearing results indicate that D-Met administered immediately before and during the first days after exposure to noise causes a dose-dependent, statistically significant attenuation of hearing thresholds shifts. The protective effect on the hearing was observed on days 7 and 14 after exposure, for D-Met at doses of 200 and 400 mg/kg body weight.

Biochemical studies on selected markers of oxidative stress indicate that D-Met administration causes a statistically significant increase in the activity of superoxide dismutases (SOD) in the tissues of the inner ear on days 7 and 14 after exposure. This effect was observed only for the highest dose of D-Met (400 mg/kg/dose). In contrast, in case of catalase, it was observed that the administration of D-met inhibits the noise-induced activity of this enzyme on day 7 after exposure. This effect was visible for D-Met at doses of 200 and 400 mg/kg body weight.

Studies on the expression of genes encoding for selected markers of oxidative stress showed a significant decrease in *Sod1* expression – gene encoding cytoplasmic SOD, on day 1 after exposure and its significant increase on day 7 after exposure, lingering up to day 14 after exposure. This effect was observed only for the highest dose of D-Met (400 mg/kg/dose). This is in accordance with the increase in the level of SOD activity in the inner ear tissues observed on analogous days. On the 7th and 14th day after the exposure, a statistically significant decrease in the expression of the *Sod2* – gene encoding the mitochondrial SOD, was observed. This effect was also observed only for the highest dose of D-Met (400 mg/kg/dose). Evaluation of catalase expression (*Cat*) at selected time points did not show significant changes for any group of mice. However, in parallel to enzymatic changes, there has been a tendency to inhibit gene expression in animals exposed to noise and treated with D-Met in comparison to animals exposed to noise and not treated D-Met.

Conclusions

1. D-Met has a protective effect on the hearing in acoustic trauma in mice.
2. The protective effect of D-Met in acoustic trauma in mice depends on the regulation of oxidative stress.
3. The increase in the activity of superoxide dismutases (SOD) in the tissues of the inner ear of mice after D-Met supplementation is associated with the increase in the expression of *Sod1* - gene encoding cytoplasmic SOD.
4. Inhibition of catalase activity in the inner ear tissues of mice after D-Met supplementation is most probably associated with inhibition of *Cat* expression, however, in the absence of statistically significant changes, this conclusion needs to be verified in subsequent studies.
5. The results obtained may be helpful in further clinical trials using D-Met in the prevention of noise induced hearing loss.

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