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Establishment of an efficacy evaluation system for treatment of hearing loss: an animal model study

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Objective

The hearing loss, characterized by difficulty to hear sounds and words, causes communication problems, leading to social isolation, disability to perceive danger, thus manifesting significant impact on social life.

Cisplatin (CDDP), a chemotherapeutic agent used to treat a variety of solid tumors, is known to cause various side effects, including ototoxicity.

In this study, a hearing loss model was prepared in C57BL/6J mice by intraperitoneal injection of CDDP. The therapeutic potential of D-methionine and Dexamethasone was tested in this model.

In addition, we report an on-going experiment of age-related hearing loss using C57BL/6J aged mice.

Summary in Japanese

難聴は音や言葉が聞こえにくい症状を指し，発症するとコミュニケーションがうまく取れず，社会的な孤立や危機察知能力の低下など，社会生活に大きな影響を及ぼす．難聴の原因は加齢や薬の副作用などが挙げられるが，現状では根本的な治療法は無く治療薬の開発が望まれている．

シスプラチンは多種多様な固形腫瘍の治療に使用される化学療法剤である． 一方で，服用により様々な副作用を呈し，その一つに聴覚毒性を引き起こすことが知られている．

今回，我々はC57BL/6Jマウスにシスプラチンを 3 mg/kgの用量で3日間腹腔内投与した後，11日間の回復期間を設け，このサイクルを3回繰り返す（シスプラチン投与：計9回）ことで難聴モデルを作製した．

このシスプラチン誘発難聴モデルにD-メチオニンとデキサメタゾンを投与したところ聴力閾値の上昇が有意に抑制されたため，聴覚毒性の軽減が示唆された．

以上の結果より，本研究で確立したシスプラチン誘発難聴モデルが難聴治療薬の効果を評価する上で有用であることが示唆された．また，現在進行している高週齢C57BL/6Jマウスを用いた加齢性難聴の知見についても報告する．

Experiment (1) Drug-induced Hearing Loss

Materials and Methods

● Animal

C57BL/6J，Male，8 weeks old

● Model preparation

CDDP was administered intraperitoneally at 3 mg/kg for 3 days followed by an 11-day recovery period.
This dosing cycle was repeated for 3 times
(CDDP was administered a total number of 9 times).

● Drug administration

▶ D-Methionine

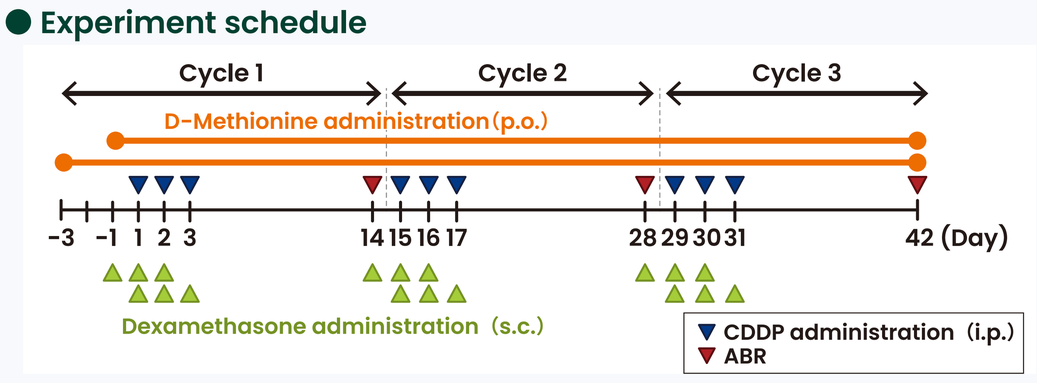
D-Methionine (300 mg/kg) was orally administered once daily from 3 days before CDDP administration or from the day of initiation of CDDP dministration to Day 42.

▶ Dexamethasone

Dexamethasone (2.5 mg/kg) was administered subcutaneously 24 hours before CDDP administration or after CDDP administration (a total of nine times).

Group	CDDP dose (mg/kg)	Drug dose (mg/kg)	Route	Number of animals
Normal	0	0	p.o.	6
Vehicle control	3	0	p.o.	6
D-Methionine-1	3	**300 mg/kg** (3 days before CDDP administration)	p.o.	6
D-Methionine-2	3	**300 mg/kg** (After CDDP administration)	p.o.	6
Dexamethasone-1	3	**2.5 mg/kg** (24 hours before CDDP administration)	s.c.	6
Dexamethasone-2	3	**2.5 mg/kg** (After CDDP administration)	s.c.	6

● Experiment schedule



● Evaluation items

- Auditory Brainstem Response (ABR)

Thresholds of ABR was measured by evoked potentials via 2 electrodes under the scalp.

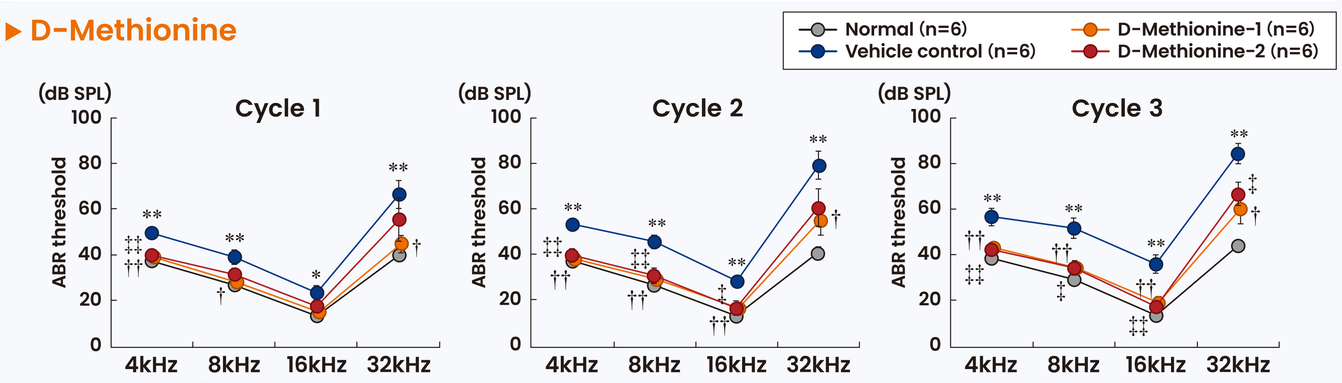
- Histopathological examination

After exsanguination under inhalation of 2 to 4% sevoflurane, the middle ear and inner ear/cochleae were collected and examined histopathologically by haematoxylin and eosin staining.

Results

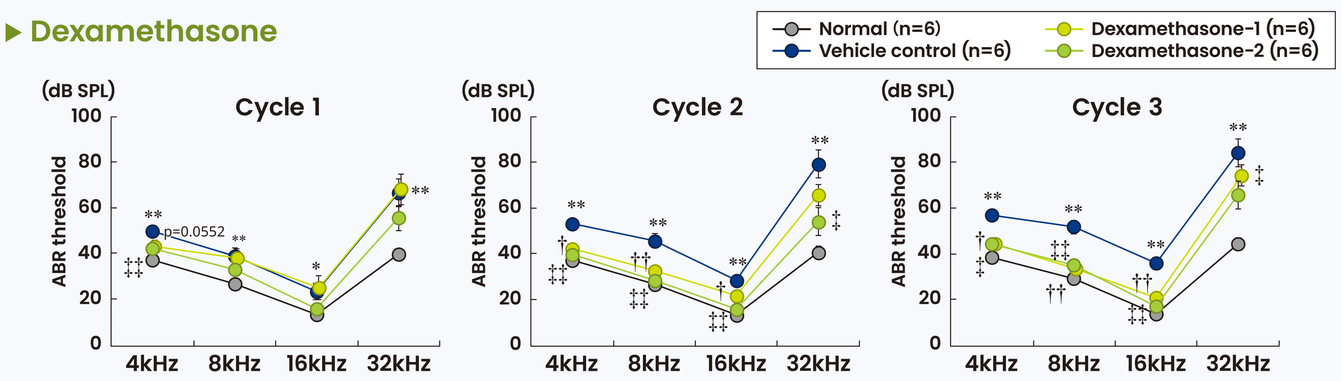
ABR (Drug-induced hearing loss)

▶ D-Methionine



Mean ± S.E.M.
*: p<0.05, **: p<0.01 normal group vs vehicle control group (Student's *t*-test)
†: p<0.05, ††: p<0.01 vehicle control group vs D-Methionine-1 group (Student's *t*-test)
‡: p<0.05, ‡‡: p<0.01 vehicle control group vs D-Methionine-2 group (Student's *t*-test

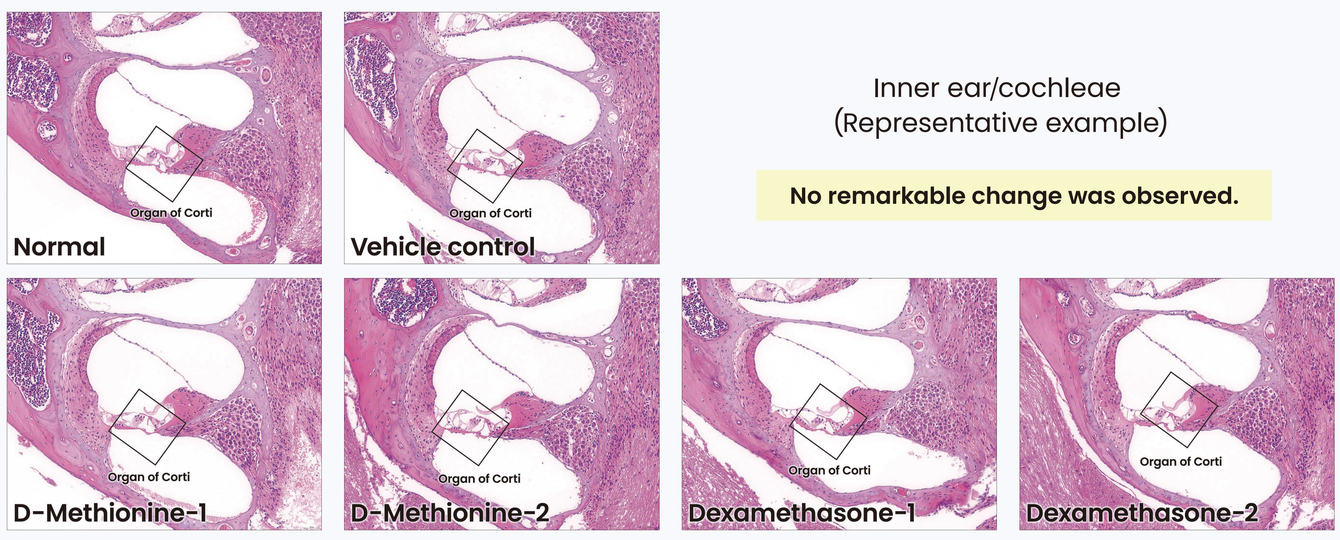
▶ Dexamethasone



Mean ± S.E.M.
*: p<0.05, **: p<0.01 normal group vs vehicle control group (Student's *t*-test)
†: p<0.05, ††: p<0.01 vehicle control group vs dexamethasone-1 group (Student's *t*-test)
‡: p<0.05, ‡‡: p<0.01 vehicle control group vs dexamethasone-2 group (Student's *t*-test)

- **Hearing threshold was increased by CDDP.**
- **D-Methionine inhibited the increase in hearing threshold.**
- **Dexamethasone inhibited the increase in hearing threshold.**

Histopathological examination (Drug-induced hearing loss)



Inner ear/cochleae
(Representative example)

No remarkable change was observed.

Experiment (2) Age-Related Hearing Loss

Materials and Methods

● Animal

C57BL/6J，Male，20-23 weeks old

● Administration

▶ Melatonin

Melatonin was administered orally once daily.

▶ Compound A

Compound A was administered orally once daily.

● Evaluation item

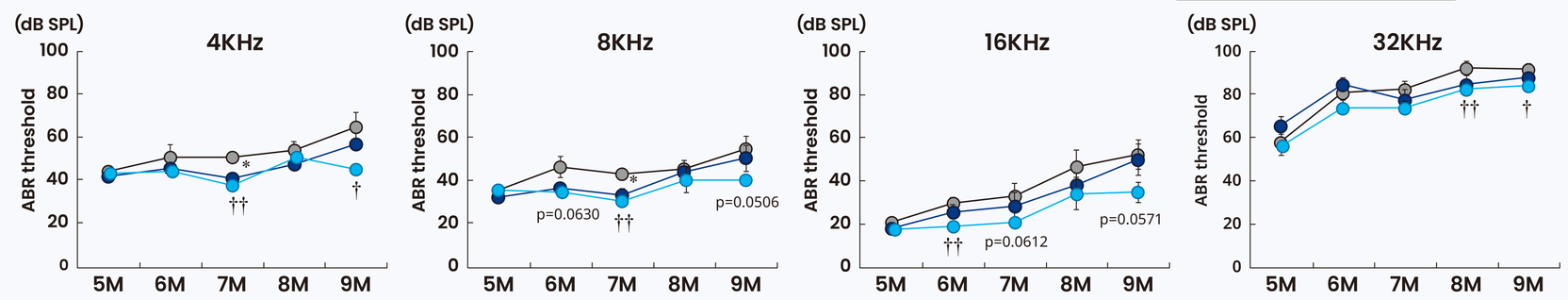
Auditory Brainstem Response (ABR)

Group	Dose (mg/kg)	Route	Number of animals
Vehicle control	0	p.o.	6
Melatonin	10	p.o.	6
Compoumd A	10	p.o.	6

Small Animal ABR Measurement System
(Tucker-Davis Technologies, inc.)

Results

ABR (Age-related hearing loss)



Mean ± S.E.M.
*: p<0.05 vehicle group vs melatonin group (Student's *t*-test)
†: p<0.05, ††: p<0.01 vehicle group vs compound A group (Student's *t*-test)

- **Hearing threshold was significant increased by the aging.**
- **Melatonin showed no effects.**
- **Compound A inhibited the increasein hearing thresholds.**

Conclusion

- CDDP induced significant increases in hearing thresholds.
- D-Methionine or Dexamethasone inhibited the increase of hearing thresholds by CDDP.
- It was confirmed that the hearing threshold increased with the aging in the experiment which carried out using aged C57BL/6J mice.
- Compound A inhibited the increase in hearing threshold although melatonin showed no obvious pharmacological effects.

In conclusion, the hearing loss model (CDDP and aging mice) were established in this study. These models were confirmed to be useful in evaluating the effects of chemicals for the treatment of hearing loss.

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