BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Nov; 17 (6): 493 - 496

Ciliary neurotrophic factor antagonizes gentamicin-induced alterations of electric potentials in auditory pathway in guinea pigs[1]

HE Cheng[2], KANG Song-Jian[3], DOU Ying[3], SHI Xian-Jun[3], WANG Cheng-Hai, AO Shi-Zhou[4], LÜ Chang-Lin

(*Department of Neurobiology, Second Military Medical University, Shanghai 200433*; [3]*Department of Physiology, Taishan Medical College, Taian 271000*; [4]*National Key Laboratory of Molecular Biology, Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS ciliary neurotrophic factor; auditory pathways; cochlear microphonic potentials; acoustic nerve; action potentials; gentamicins

AIM: To study the effects of ciliary neurotrophic factor (CNTF) on the expressions of gentamicin ototoxicity in guinea pigs. **METHODS:** The auditory function of pigmented guinea pigs was examined using auditory brainstem response (ABR), cochlea microphonic potential (CM), and action potential of auditory nerve (AP). **RESULTS:** In animals injected gentamicin (80 $mg \cdot kg^{-1} \cdot d^{-1}$, im), ABR threshold began to elevate on d 20, and prolongations of ABR wave I, IV and the I—IV interpeak latencies were observed. The animals treated with gentamicin for 30 d displayed lower amplitudes of CM and AP (N1) than the controls. CNTF (0.44 $mg \cdot kg^{-1} \cdot d^{-1}$, sc) inhibited the gentamicin-induced elevation of ABR thresholds, the prolongation of ABR wave I, IV and the I—IV interpeak latencies, and the decreases in amplitudes of CM and AP (N1). **CONCLUSION:** CNTF attenuated the gentamicin-elicited auditory impairment in guinea pigs.

Gentamicin produces degeneration of sensory hair cells in the organ of Corti[1] and induces alteration in central auditory pathways[2,3,4]. It is important to investigate further preventive measures to reduce the severity and frequency of the ototoxicity.

Ciliary neurotrophic factor (CNTF) was originally identified and partially purified from embryonic chick eye tissues. CNTF supported the survival of sensory, sympathetic, and motor neurons[5], and promoted neurite outgrowth of statoacoustic ganglia combined with otocyst derived factor[6]. But no report on the effect of CNTF on the auditory impairment caused by gentamicin was found. In the present study, the effect of recombinant CNTF on the expression of gentamicin ototoxicity was examined.

MATERIALS AND METHODS

Reagents The recombinant human CNTF was prepared according to previous report[7]. Its bioactivity was 1.0 neurotrophic unit per ng protein. It was kept at −20 ℃, and thawed before use. The gentamicin sulfate was purchased from Yongnin Pharmaceutical Inc.

Animals Pigmented guinea pigs weighting 277 ± *s* 17 g and showing normal Preyer's reflex were housed at 22.0 ± 0.5 ℃ with an alternating 12 h light-dark cycle (7:00—19:00). Food and water were given *ad lib*. Animals were divided into 5 groups: 1) im gentamicin 80 $mg \cdot kg^{-1} \cdot d^{-1}$ for 30 d; 2) sc CNTF 0.44 $mg \cdot kg^{-1} \cdot d^{-1}$ after each gentamicin; 3) sc CNTF 0.11 $mg \cdot kg^{-1} \cdot d^{-1}$ after gentamicin; 4) and 5) sc CNTF 0.44 $mg \cdot kg^{-1} \cdot d^{-1}$ and normal saline, respectively, without gentamicin.

Auditory brainstem responses The recording electrodes were inserted subcutaneously below the ipsilateral pinna (reference electrode) and the vertex (active electrode). The ground electrode was inserted subcutaneously below the contralateral pinna. The auditory brainstem responses (ABR) to alternating polarity click stimuli delivered to each ear separately (earphone against the ear) 10 times $\cdot s^{-1}$, at intensity from 70 dB peSPL down to ABR threshold (the lowest intensity elicited a distinct wave IV) was recorded. Recorded electric activity was bandpass filtered (80 − 3000 Hz), amplified and averaged ($n = 500$) by a YJC-A evoked response instrument. ABR threshold, peak latencies of wave I and IV, and the interpeak latency of I − IV (brainstem transmission time, BTT) were analyzed.

Cochlear potentials Three days after the final

[1] Project supported by the National Natural Science Foundation of China, No 39500044

[2] Correspondence to Dr HE Cheng.

Received 1995-09-25 Accepted 1996-06-05

medication, the animals were anesthetized with ip urethane 1.0 g·kg^{-1}. The recording electrode was implanted in the scala tympani and the reference electrode was implanted in the superficial masseter muscle. The cochlear microphonic potential (CM) and action potential of auditory nerve (AP) was recorded in response to the click, at intensity of 20 or 40 dB peSPL, amplified and monitored on a wave analyzer. All measurements were made in a sound treated and electrically shielded room.

RESULTS

CM The amplitudes of CM in the animals given gentamicin (GE) 80 mg·kg^{-1}·d^{-1} for 30 d were lower than those treated with saline. The amplitudes in the animals injected sc CNTF 0.44 mg·kg^{-1}·d^{-1} after gentamicin were higher than those given gentamicin alone. This CNTF attenuated the reduction of the amplitudes caused by gentamicin. However, sc CNTF 0.11 mg·kg^{-1}·d^{-1} failed to attenuate the reduction of the amplitudes. No difference was found between CNTF 0.44 mg·kg^{-1}·d^{-1} group and the normal saline group (Tab 1).

AP The GE group displayed a lower AP(N1) amplitudes than the saline group. The animals injected sc CNTF 0.44 mg·kg^{-1}·d^{-1} after gentamicin exibited higher AP(N1) amplitudes than those given gentamicin alone. This CNTF attenuated the reduction of the AP(N1) amplitudes in the animals treated with gentamicin, but did not influence the amplitudes of normal animals. There were no significant differences between the GE +0.11 mg·kg^{-1} group and the GE group (Tab 1).

ABR ABR was measured prior to the start of the study and then again on d 5, 10, 15, 20, 25, and 30 after beginning of treatment. ABR thresholds were comparable in all groups at the beginning of the study. Animals received gentamicin 80 mg·kg^{-1}·d^{-1} for 30 d showed a progressive hearing loss. The threshold in GE group began to elevate on d 20 *vs* saline group. Animals treated with CNTF 0.44 mg·kg^{-1}·d^{-1} and gentamicin showed a reduction of the threshold shift. The GE + 0.11 mg·kg^{-1} group showed a slightly lower ABR threshold *vs* gentamicin group ($P>0.05$). There were no significant differences between animals treated with saline and CNTF alone (Fig 1).

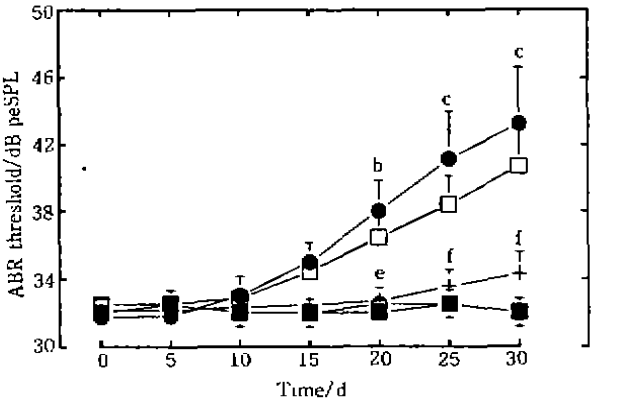

Fig 1. ABR threshold. (○) **Saline,** $n=5$. (●) **GE,** $n=10$. (+) **GE + CNTF 0.44 mg·kg^{-1},** $n=14$. (□) **GE + CNTF 0.11 mg·kg^{-1},** $n=14$. (■) **CNTF 0.44 mg·kg^{-1},** $n=5$. $^{b}P<0.05$, $^{c}P<0.01$ *vs* **saline;** $^{e}P<0.05$, $^{f}P<0.01$ *vs* **GE.**

The latencies of wave I in GE group on d 25 and 30 were longer than that in animals receiving saline. CNTF 0.44 mg·kg^{-1}·d^{-1} inhibited the prolongation of wave I latencies (Fig 2A). The latencies of wave IV in GE group on d 20, 25, and 30 were longer than that in the saline group. The prolongation of wave IV latencies induced by

Tab 1. Amplitudes (μV) of CM and AP (N1). $^{b}P<0.05$, $^{c}P<0.01$ *vs* **saline group;** $^{e}P<0.05$, $^{f}P<0.01$ *vs* **GE group.**

Group	n	CM		AP	
		20 dB	40 dB	20 dB	40 dB
Saline	5	348 ± 37	505 ± 42	542 ± 39	854 ± 47
CNTF 0.44 mg·kg^{-1}	5	352 ± 27	503 ± 37	550 ± 39	852 ± 46
GE	10	163 ± 35^{c}	280 ± 59^{c}	344 ± 54^{b}	567 ± 93^{b}
GE + CNTF 0.44 mg·kg^{-1}	14	328 ± 31^{f}	496 ± 48^{f}	528 ± 54^{e}	828 ± 74^{e}
GE + CNTF 0.11 mg·kg^{-1}	14	195 ± 24	303 ± 40	372 ± 37	584 ± 46

gentamicin was inhibited by the high or low dose CNTF (Fig 2B). The BTT in the gentamicin group on d 30 was longer than that in the saline group. Animals received gentamicin and CNTF 0.44 mg·kg^{-1}·d^{-1} showed normal BTT values (Fig 2C). There were no significant difference in the latencies of wave I and wave IV, and the BTT between CNTF group and saline group.

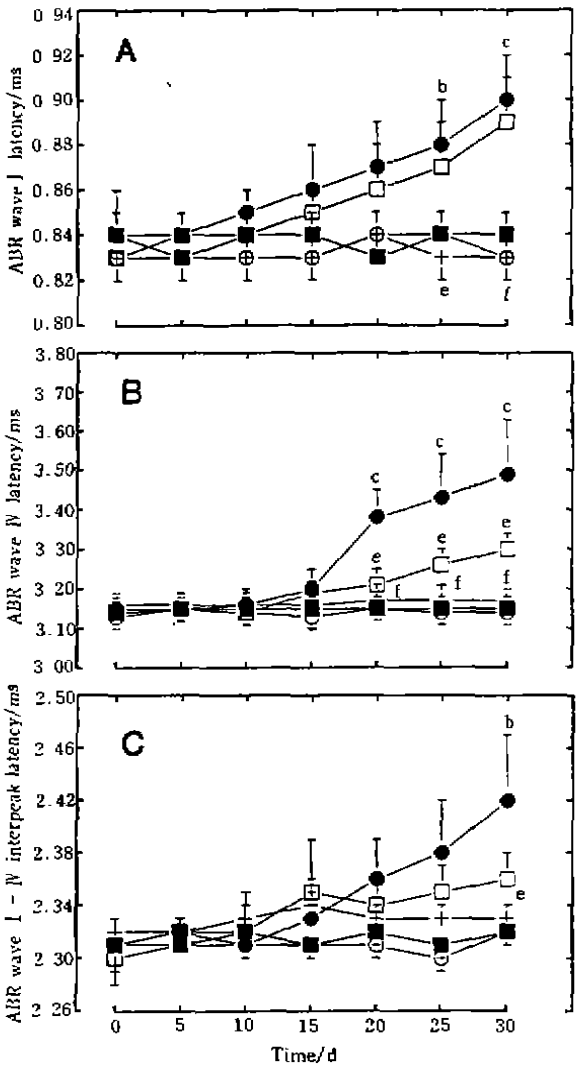

Fig 2. Latencies of ABR wave I (A), wave IV (B) and the I−IV interpeak latency (C). (○) Saline, $n = 5$. (●) GE, $n = 10$. (+) GE + CNTF 0.44 mg·kg^{-1}, $n = 14$. (□) GE + CNTF 0.11 mg·kg^{-1}, $n = 14$. (■) CNTF 0.44 mg·kg^{-1}, $n = 5$. $^bP<0.05$, $^cP<0.01$ *vs* saline. $^eP<0.05$, $^fP<0.01$ *vs* GE.

DISCUSSION

The gentamicin-induced damage of sensory hair cells and auditory nerve fibers in corti can result in declines in the amplitudes of the CM and AP (N1)[8,9]. The impairment in corti can also cause the elevation of ABR threshold and the prolongation of latencies of wave I and wave IV as well[2]. In present study, subcutaneous administration of recombinant CNTF inhibited the gentamicin-induced the decrease in amplitude of CM and AP(N1), the elevation of ABR thresholds, and the prolongation of ABR wave I, IV latencies. These results indicated that CNTF co-administration can attenuate the impairment of hair cells and auditory nerve fibers in corti. Another cause of the alteration in ABR is the cumulative toxicity of the aminoglycoside occurred in auditory nervous system. The observation that CNTF inhibited the prolongation of BTT suggested that CNTF could protect partial central auditory pathway against the toxicity of gentamicin. It was shown that the daily subcutaneous injection of CNTF (0.11 mg·kg^{-1}) was much less effective than the high dosage (0.44 mg·kg^{-1}). This could be due to the short life of CNTF in the circulation[10,11]. A relative high pharmacological concentration of CNTF in circulation was needed for its attenuation effect on cumulative toxicity of gentamicin.

Besides inducing damage of sensory hair cells and auditory nerves, the long treatment of gentamicin can cause the impairment of the vestibule, weight loss, and nephrotoxicity[4]. The signs of vestibular toxicity such as the stereotype and abnormal gait were observed in some animal treated with gentamicin, while in the high dose CNTF treatment group few animal exhibited these signs. With respect to body weight, the groups with gentamicin and the groups with CNTF and gentamicin had lower weight than the controls (unpublished data). The effects of CNTF on the vestibular and other toxicities should be clarified by further studies.

The production of neurotrophic factors in the target organs of primary sensory neurons is believed to be important in maintaining neuron survival. The mRNA encoding two members of the neurotrophin gene family, brain derived neurotrophic factor and neurotrophin-3, were synthesized by inner and outer hair cells of Corti[12]. Genes

encoding neurotrophin receptors were expressed in the neurons of VⅢth ganglion[13]. Significant levels of CNTF mRNA in the adult rat central nervous system (CNS) were found in the brain stem, cerebellum, midbrain, and thalamus/hypocampus[14]. The CNTF receptor α subunit (CNTF binding protein) was quite widely expressed in the both the developing and adult CNS[15]. It is not clear whether the CNTF and its receptor were existed in inner ear, but CNTF, combined with otocyst-derived factor, was found to promote the outgrowth of cultured statoacoustic ganglia[6]. The mechanism that CNTF attenuates gentamicin-induced ototoxicity shoud be further investigated.

ACKNOWLEDGMENTS To Dr George D Yancopoulos for the generous gift of human CNTF cDNA.

REFERENCES

1 Matz GJ. Aminoglycoside ototoxicity.
Am J Otolaryngol 1986; 7: 117 - 9

2 Hotz MA, Allum JH, Kaufmann G, Follath F, Pfaltz CR. Shifts in auditory brainstem response latencies following plasma-level-controlled aminoglycoside therapy.
Eur Arch Otorhinolaryngol 1990; 247: 202 - 5

3 Hodges GR, Watanabe I, Singer P, Rengachary S, Reeves D, Jusetesen DR, *et al*. Central nervous system toxicity of intraventricular administered gentamicin in adult rabbits.
J Infect Dis 1981; 143: 148 - 55.

4 Sullivan MJ, Rarey KE, Conolly RB. Comparative ototoxicity of gentamicin in the guinea pig and two strains of rats.
Hear Res 1987; 31: 161 - 8

5 Ip NY, Yancopoulos GD. Ciliary neurotrophic factor and its receptor complex. Prog Growth Factor 1992; 4: 139 - 55.

6 Bianchi LM, Cohan CS. Effect of the neurotrophins and CNTF on developing statoacoustic neurons: comparison with an otocyst-derived factor. Dev Biol 1993; 159: 353 - 65.

7 He C, Chen JY, Ao SZ, Lu CL. Preparation and a structure-function analysis of human ciliary neurotrophic factor
Neurosci Res 1995; 23: 327 - 33.

8 Prazma J, Ferguson SD, Kidwell SA, Garrison HG, Drake A, Fischer J. Alteration of aminoglycoside antibiotic ototoxicity by hyper- and hypohydration.
Am J Otolaryngol 1981; 2: 299 - 306

9 Lenoir M, Marot S, Uziel A. Comparative ototoxicity of four aminoglycosidic antibiotics.
Acta Otolaryngol (Suppl) (Stockh) 1983; 405: 1 - 16.

10 Dittrich F, Thoenen H, Sendtner M. Ciliary neurotrophic factor: pharmacokinetics and acute phase response.
Ann Neurol 1994; 35: 151 - 63.

11 Helgren ME, Squinto SP, Davis HL, Parry DJ, Boulton TG, Heck CS, *et al*. Trophic effect of ciliary neurotrophic factor on denervated skeletal muscle. Cell 1994; 76: 493 - 504

12 Wheeler EF, Bouthwell M, Schecterson LC, von Bartheld CS. Expression of BDNF and NT-3 mRNA in hair cells of the organ of Corti: quantitative analysis in developing rats
Hear Res 1994; 73: 46 - 56.

13 Schecterson LC, Bouthwell M. Neurotrophin and neurotrophin receptor mRNA expression in developing inner ear.
Hear Res 1994; 73: 92 - 100

14 Ip NY, Wiegand SJ, Morse J, Rudge JS. Injury-induced regulation of ciliary neurotrophic factor messenger-RNA in the adult rat brain. Eur J Neurosci 1993; 5: 25 - 33.

15 Ip NY, McClain J, Barrezueta NX, Aldrich TH, Pan L, Li Y, *et al*. The α-component of the CNTF receptor is required for signaling and defined potential CNTF targets in the adult and during development. Neuron 1993; 10: 98 - 102.

493-496

睫状神经营养因子抑制庆大霉素引起的豚鼠听觉生物电的改变[1]

何 成[2], 康颂建[3], 窦 颖[3], 史献君[3], 王成海, 敖世洲[4], 路长林 (第二军医大学神经生物学教研室, 上海 200433; [3]泰山医学院生理学教研室, 泰安 271000; [4]中国科学院上海生物化学研究所国家分子生物学重点实验室, 上海 200031, 中国)

关键词 睫状神经营养因子; 听觉通路; 耳蜗微音器电位; 听神经; 动作电位; 庆大霉素

A **目的**: 研究睫状神经营养因子(CNTF)对庆大霉素(GE)所致豚鼠耳毒性的影响 **方法**: 以听觉脑干诱发电位(ABR)、耳蜗微音器电位(CM)和听神经动作电位(AP)为指标, 测定豚鼠听力功能. **结果**: 豚鼠给予 GE (80 mg·kg^{-1}·d^{-1}, im), 20 d 后可见 ABR 阈值显著升高, ABR 波 I 和波 IV 波峰潜伏期和波 I－波 IV 波峰间期显著延长; 30 d 后, CM 波和 AP(N1)波波幅明显降低 CNTF (0.44 mg·kg^{-1}·d^{-1}, sc)能够抑制 GE 引起的 ABR 阈值升高; 能阻止 GE 引起的 ABR 波 I 和波 IV 波峰潜伏期和波 I－波 IV 波峰间期延长; 能够减轻 CM 波和 AP(N1)波波幅降低. **结论**: CNTF 能够对抗 GE 引起的豚鼠听力损伤

R987 R965.1