Involvement of Poly(ADP-Ribose) Synthetase in Acoustic Trauma of The Cochlea

KEIJI TABUCHI, TOMOHUMI HOSHINO, HIDEKAZU MURASHITA, KEIKO OIKAWA, ISAO UEMAETOMARI, BUNGO NISHIMURA, TADAMICHI TOBITA and AKIRA HARA

Department of Otolaryngology, Institute of Clinical Medicine, University of Tsukuba, Tsukuba 305-8575

TABUCHI, K., HOSHINO, T., MURASHITA, H., OIKAWA, K., UEMAETOMARI, I., NISHIMURA, B., TOBITA, T. and HARA, A. *Involvement of Poly(ADP-ribose) Synthetase in Acoustic Trauma of The Cochlea*. Tohoku J. Exp. Med., 2003, **200** (4), 195–202 — We investigated effects of poly(ADP-ribose) synthetase (PARS) inhibitors on acoustic trauma. Albino guinea pigs were intravenously given 3-aminobenzamide, nicotinamide or 3-aminobenzoic acid (an inactive analog of 3-aminobenzamide) just prior to exposure to a 2 kHz pure tone of 120 dB sound pressure level (SPL) for 10 minutes. The threshold of the compound action potential (CAP) and the amplitude of distortion-product otoacoustic emissions (DPOAEs) were measured before and 4 hours after the acoustic overexposure. Statistically significant decreases in the CAP threshold shifts and significant increases in the DPOAE amplitudes were observed 4 hours after the acoustic overexposure in the animals treated with 3-aminobenzamide or nicotinamide, whereas 3-aminobenzoic acid did not exert any protective effect. These results strongly suggest that excessive activation of PARS is involved in generation of the acoustic trauma. ——— Acoustic trauma; Cochlea; Poly(ADP-ribose) synthetase (PARS)

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Poly(ADP-ribose) synthetase (PARS) is a nuclear enzyme that forms a polymer of ADP-ribose on nuclear acceptor molecules such as histones, topoisomerase and, predominantly, PARS itself. Recently, its role under pathological conditions, particularly involving the reactive oxygen species and nitric oxide (NO)-induced damage, has come to interest more researchers. It has been demonstrated that

reactive oxygen species (Schraufstatter et al. 1986; Hyslop et al. 1988), NO (Heller et al. 1995; Cookson et al. 1998) and peroxynitrite (Szabo et al. 1996; Cookson et al. 1998) cause DNA strand breaks, which, in turn, activate PARS to utilize nicotinamide adenine dinucleotide (NAD). Since NAD is essential to mitochondrial electron transport, NAD depletion by excessive activation of PARS interferes with production

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Address for reprints: Keiji Tabuchi, Department of Otolaryngology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan.

e-mail: ktabuchi@md.tsukuba.ac.jp

of adenosine triphosphate (ATP) to ultimately lead to cell death (Schraufstatter et al. 1986; Hyslop et al. 1988; Heller et al. 1995; Szabo et al. 1996).

Recently, it has been demonstrated that intense sound exposure increases generation of hydroxyl radicals (Ohlemiller et al. 1999). In addition, the protective effects of free radical scavengers on acoustic trauma have been demonstrated by many researchers (Seidman et al. 1993; Yamasoba et al. 1999). These findings strongly suggest that activation of PARS by free radicals generated during and after noise exposure may also play an important role in this type of injury. However, to our knowledge, the involvement of PARS in acoustic trauma has never been examined. In order to prove the involvement of PARS, the effect of 3-aminobenzamide (3AB) and nicotinamide (NA), inhibitors of PARS, and 3-aminobenzoic acid (3ABA), an inactive analog of 3-aminobenzamide (Rankin et al. 1989; Banasik et al. 1992; Thiemermann et al. 1997), on the cochlear dysfunction induced by acoustic trauma was investigated.

MATERIALS AND METHODS

Animals

Studies were performed on 46 albino guinea pigs weighing 250–350 g with a normal Preyer pinna reflex. Anesthesia was induced by pentobarbital sodium (28 mg/kg ip., Nembutal®, Abbott, North Chicago, IL, USA). A supplementary dose of pentobarbital sodium was given at appropriate intervals. A cannula was inserted in the external jugular vein for administration of drugs. Body temperature was maintained at

$37 \pm 1^\circ\text{C}$ using a heating pad. All experimental procedures used in this study were approved by the Animal Experiment Committee at University of Tsukuba under the Guidelines of the Declaration of Helsinki.

Compound action potential measurement

Methods for measuring compound action potential (CAP) were described elsewhere (Okubo et al. 1995; Tabuchi et al. 2001b). In brief, CAP was recorded at the bony edge of the round window by an Ag/AgCl electrode. The reference electrode was placed on the neck muscles. In order to evoke CAP, tone bursts of pure tones from 2 to 16 kHz (rise and fall times, 1 milliseconds; duration, 10 milliseconds) were delivered in an open field system. Thirty-two, 64 or 128 responses were averaged, and the sound pressure level necessary to evoke a CAP of $10 \mu\text{V}$ was defined as the threshold.

Distortion-product otoacoustic emissions measurement

Because our recording methods for distortion-product otoacoustic emissions (DPOAEs) have been described elsewhere (Okubo et al. 1995; Tabuchi et al. 2001a), only a brief summary will be given here. Two primary tones were delivered to the emitters contained within a custom-made ear probe. The frequencies and levels of the primary tones are shown in Table 1. Geometric means (GM) of f_1 and f_2 were 4, 6 and 8 kHz, and f_2/f_1 was fixed at 1.2, the most appropriate ratio to evoke the maximum response (Okubo et al. 1995). DPOAEs were recorded by a microphone (EK-3024, Knowles, Itasca, IL, USA) inside the ear

TABLE 1. *Frequencies and levels of f_1 and f_2*

GM (Hz)	f_1 (Hz/sBSPL)	f_2 (Hz/dBSPL)	$2f_1-f_2$ (Hz)
4000	3650/60	4375/50	2925
6000	5475/50	6575/40	4375
8000	7300/50	8800/40	5800

GM, geometric means.

probe and processed by a spectrum analyzer (CF-350, Ono Sokki, Tokyo).

Experimental design

The effects of intravenous administration of 10 mg/kg 3AB ($n=3$), 20 mg/kg NA ($n=3$) or 10 mg/kg 3ABA ($n=3$) on the CAP thresholds or DPOAE amplitudes of untreated control animals were examined. The CAP thresholds or DPOAEs 4 hours after the administration were compared with the pre-administration values.

In order to induce acoustic trauma, the left ear was exposed to 2 kHz pure tone of 120 dB SPL through the hollow ear bar for 10 minutes. The animals subjected to acoustic trauma were divided into the following 4 groups:

(1) 3AB-given group ($n=16$): Each animal in this group was intravenously given 1 ($n=5$), 5 ($n=5$) or 10 ($n=6$) mg/kg 3AB (dissolved in 3 ml/kg physiological saline solution [PSS]) just before the onset of acoustic exposure.

(2) NA-given group ($n=11$): Each animal in this group was intravenously given 10 ($n=5$) or 20 ($n=6$) mg/kg NA (dissolved in 3 ml/kg PSS) just before the onset of acoustic exposure.

(3) 3ABA-given group ($n=5$): Each ani-

mal in this group was intravenously given 10 mg/kg 3ABA (dissolved in 3 ml/kg PSS) just before the onset of acoustic exposure.

(4) PSS-given group ($n=5$): Each animal in this group received 3 ml/kg PSS intravenously just before the onset of acoustic exposure.

Shifts in the CAP thresholds and DPOAE amplitudes from the pre-exposure values were compared between the compound- and the PSS-given groups 4 hours after the acoustic over-exposure.

Statistics

All data were expressed as mean $\pm$ S.E.M. Comparisons of CAP or DPOAEs were evaluated by two-way and one-way ANOVA followed by Scheffe test as required. A p -value of less than 0.05 was considered statistically significant.

RESULTS

Figs. 1A, 1B and 1C show the CAP thresholds before and 4 hours after the administration of 10 mg/kg 3AB, 20 mg/kg NA and 10 mg/kg 3ABA, respectively ($n=3$ in each group). Intravenous administration of these compounds did not significantly affect the CAP thresholds (two-way and one-way ANOVA). As shown in

Fig. 1. CAP thresholds before and 4 hours after administration of 10 mg/kg 3-aminobenzamide (A, $n=3$), 20 mg/kg nicotinamide (B, $n=3$) or 10 mg/kg 3-aminobenzoic acid (C, $n=3$). The pre- and post-administration values are indicated with squares and circles, respectively. The CAP thresholds were not affected significantly by administration of these drugs (two-way and one-way ANOVA, $p>0.05$).

Fig. 2. DPOAE amplitudes before and 4 hours after administration of 10 mg/kg 3-aminobenzamide (A, $n=3$), 20 mg/kg nicotinamide (B, $n=3$) or 10 mg/kg 3-aminobenzoic acid (C, $n=3$). The pre- and post-administration values are indicated with squares and circles, respectively. The DPOAE amplitude was not affected significantly by administration of these drugs (two-way and one-way ANOVA, $p>0.05$).

Fig. 3. Threshold shifts in CAP 4 hours after the acoustic overexposure. Closed circles indicate the PSS group. * $p<0.05$ compared to the PSS group with one-way ANOVA followed by Scheffe test.

A: 3-aminobenzamide (3AB) group. Squares, triangles and open circles indicate the 1-, 5- and 10-mg/kg 3AB groups, respectively.

B: Nicotinamide (NA) group. Squares and triangles, indicate the 10- and 20-mg/kg NA groups, respectively.

C: 3-aminobenzoic acid (3ABA) group. Open circles indicate the 10-mg/kg 3ABA group.

Figs. 2A, B and C, the amplitude of DPOAEs was not significantly affected by these compound, either ($n=3$ in each group, two-way and one-way ANOVA).

Fig. 3 shows the CAP threshold shifts in 3AB-, NA- and 3ABA-given animals 4 hours

after the acoustic overexposure. Although 1 ($n=5$) or 5 ($n=5$) mg/kg 3AB did not affect the CAP threshold shifts, 10 ($n=6$) mg/kg 3AB significantly decreased the CAP threshold shifts (two-way ANOVA: $F=7.4$, $p<0.001$, Scheffe test: $p<0.001$ in the 10 mg/kg subgroup, $p>$

Fig. 4. Amplitude reduction in DPOAEs (dB re pre-exposure level) 4 hours after the acoustic overexposure. Closed circles indicate the PSS group. * $p < 0.05$ compared to the PSS group with one-way ANOVA followed by Scheffe test.

A: 3-aminobenzamide (3AB) group. Squares, triangles and open circles indicate the 1-, 5- and 10-mg/kg 3AB groups, respectively.

B: Nicotinamide (NA) group. Squares and triangles, indicate the 10- and 20-mg/kg NA groups, respectively.

C: 3-aminobenzoic acid (3ABA) group. Open circles indicate the 10-mg/kg 3ABA group.

0.05 in the 1 or 5 mg/kg subgroup, one-way ANOVA and Scheffe test: $p < 0.05$ at 2 and 4 kHz in the 10 mg/kg subgroup) (Fig. 3A). In addition, although 10 ($n=5$) mg/kg NA did not affect the CAP threshold shifts, 20 ($n=5$) mg/kg NA significantly ameliorated the cochlear dysfunction (two-way ANOVA: $F=33.2$, $p < 0.001$, Scheffe test: $p < 0.001$ in the 20 mg/kg subgroup, $p > 0.05$ in the 10 mg/kg subgroup, one-way ANOVA and Scheffe test: $p < 0.05$ at 2, 4, 8 and 16 kHz in the 20 mg/kg subgroup) (Fig. 3B). However, 3ABA, an inactive analogue of 3AB, did not affect the CAP threshold shifts at 10 mg/kg ($n=6$, two-way and one-way ANOVA) (Fig. 3C).

Fig. 4 shows the amplitude reduction in DPOAEs in reference to the pre-exposure level 4 h after the acoustic overexposure. Although 1 ($n=5$) or 5 ($n=5$) mg/kg 3AB did not affect the DPOAE amplitudes, 10 ($n=6$) mg/kg 3AB significantly ameliorated the reduction of the DPOAE amplitude by acoustic overstimulation (two-way ANOVA: $F=3.2$, $p < 0.05$, Scheffe test: $p < 0.05$ in the 10 mg/kg subgroup, $p > 0.05$

in the 1 or 5 mg/kg subgroup, one-way ANOVA and Scheffe test: $p < 0.05$ at 8 kHz in the 10 mg/kg subgroup) (Fig. 4A). NA also exhibited the protective effect against acoustic trauma at 20 mg/kg (two-way ANOVA: $F=16.7$, $p < 0.001$, Scheffe test: $p < 0.001$ in the 20 mg/kg subgroup, $p > 0.05$ in the 10 mg/kg subgroup, one-way ANOVA and Scheffe test: $p < 0.05$ at 4, 6 and 8 kHz in the 20 mg/kg subgroup) (Fig. 4B). In contrast to the findings observed in the animals treated with PARS inhibitors, there was no statistically significant difference in DPOAEs between the 3ABA-given and the PSS-given groups (two-way and one-way ANOVA) (Fig. 4C).

DISCUSSION

Since PARS catalyzes the transfer of ADP ribose moieties from NAD to nuclear acceptor molecules, excessive activation of PARS depletes the intracellular concentration of NAD and may lead to cell death (Schraufstatter et al. 1986; Heller et al. 1995; Szabo et al. 1996; Takasawa and Okamoto 2002). Reactive oxygen

species (Schraufstatter et al. 1986; Hyslop et al. 1988), NO (Heller et al. 1995; Cookson et al. 1998) and peroxynitrite (Szabo et al. 1996; Cookson et al. 1998) have been proposed as powerful triggers of DNA strand breaks and consequent excessive activation of PARS. It has been demonstrated that PARS inhibition ameliorated various CNS (central nervous system) diseases, e.g., Parkinson's disease, brain trauma and ischemia-reperfusion injury (Mikael et al. 1997; Allen et al. 1999; LaPlaca et al. 2001). Recent studies suggest that reactive oxygen species, such as superoxide and hydroxyl radical contribute to acoustic trauma (Seidman et al. 1993; Ohlemiller et al. 1999; Yamasoba et al. 1999; Hara et al. 2000). These findings strongly suggest that activation of PARS by free radicals may also play an important role in this type of injury.

One of the major findings in the present study was the significant protection of cochlear function against acoustic trauma observed in the 3AB- and the NA-given animals. To our knowledge, this finding demonstrated for the first time that PARS plays a deleterious role in acoustic trauma.

Several lines of the evidence suggest that damage to the outer hair cells (OHCs) is the initial effect of acoustic overstimulation (Davis et al. 1989; Hamernik et al. 1989; Boettcher et al. 1992). DPOAEs are supposed to be closely related to an active process in the organ of Corti and are a useful test of OHC function (Whitehead et al. 1992). In the present study, 3AB and NA significantly increased the DPOAE amplitude 4 hours after noise exposure. Based on this finding, it is highly likely that PARS inhibitors protect OHCs to alleviate CAP threshold shifts after acoustic trauma.

One may argue whether the protective effects of PARS inhibitors demonstrated in the present study are due to its specific effect. In the present study, although 3AB and NA significantly ameliorated the cochlear dysfunction after noise exposure, 3ABA, an inactive ana-

logue of 3AB that does not inhibit PARS activity (Rankin et al. 1989; Banasik et al. 1992; Thiernemann et al. 1997), did not affect the cochlear function after acoustic trauma. This finding strongly suggests that the protective effects of 3AB and NA observed in the present study were due to PARS inhibition.

A previous study in our laboratory has demonstrated that the present model of acoustic trauma induces the permanent threshold shift (PTS) (Takahashi et al. 1996). Because we examined cochlear function 4 h after the acoustic overexposure in the present study, it was not clarified whether PARS inhibitors ameliorated PTS after acoustic injury. However, we confirmed in the mouse model of acoustic injury that 3AB significantly reduced PTS and loss of OHCs 2 week after acoustic overexposure of 128 dB SPL for 4 hours (Tabuchi et al. unpublished data).

In conclusion, 3AB and NA, PARS inhibitors, exerted protective effects against cochlear injury induced by acoustic trauma, although 3ABA did not exhibit such effects. These findings suggest that excessive activation of PARS may play an important role in the generation of acoustic trauma.

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