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Effect of ascorbic acid on the numerical hair cell loss in noise exposed guinea pigs

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Two groups of guinea pigs were exposed to 1/3 octave band noise centered at 4 kHz, 113–118 dB SPL, for 2 h. The animals of the first group were treated with ascorbic acid (AA), 0.5 mg per 1 g of body mass injected intraperitoneally before noise exposure. The second group (control) was exposed without being treated. By means of the surface specimen method and consequent assessment of numerical atrophy of cochlear hair cells it was found that application of ascorbic acid before the noise exposure resulted in a lower or no loss of hair cells especially within the respective frequency segment of the basilar membrane. Possible protective effect of AA and/or the negative effect of hypovitaminosis "C" are discussed.

Cochlear hair cell loss; Noise, effect of; Ascorbic acid

As explicitly stated by Kellerhals (1972), it is hardly possible to find a successful prescription for resuscitation of irreversibly damaged cochlear hair cells. The only possibility is to hold up the slow degenerative process caused by metabolic fatigue or by mechanical stress (Stockwell et al., 1969; Thorne and Gavin, 1985) by improving the metabolic balance of exhausted cells by administration of several chemical compounds which, it is supposed, are involved in cochlear metabolism.

Several authors have suggested that supplemental vitamins, such as vitamin A or B-complex in massive doses, may prevent ototoxicity of some aminoglycoside antibiotics (Ukleja, 1967; Darrouzet, 1963) or reverse sensorineural hearing loss and improve tinnitus (Baron, 1951). Some investigators have also claimed that free-radical scavengers (to which ascorbic acid belongs) should bear some prophylactic effect after noise exposure or on kanamycin-induced ototoxicity (Pierson and

Møller, 1981). But according to others (Chole and Quick, 1976; Chole, 1980), the role of vitamins (especially vitamin A) in the auditory apparatus remains unclear and also evidence of the protective effect of supplemental doses of these compounds against noise-induced hearing loss is still inconclusive (Ward and Glorig, 1960; Ivstam, 1960).

In light of the fact that some possibility to stimulate the resistance against metabolic exhaustion caused by acoustic stress may exist, we decided to use and test ascorbic acid as a protective factor against noise induced hair cell loss. We considered, above all, that according to Selye (1976) one of the most characteristic consequences of exposure to acute stress is the loss of ascorbic acid from the adrenal cortex, and that decreased ascorbic acid concentrations in the blood were described even after excessive acoustic stimulation (Geber et al., 1966).

Ten healthy pigmented guinea pigs (six weeks old, body mass approx. 300 g, manifesting Preyer's reflex) were randomly divided into two groups of 5 individuals. The animals of the first group were treated with a solution of ascorbic acid (0.5 mg

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AA per 1 g body mass) administered intraperitoneally 48 h, 24 h and 5 min before noise exposure. The other group of animals served as a control group. The amount of vitamin C used in our experiment was not considered to be toxic (to cause a calcium metabolism failure). It has been already reported by Sorenson et al. (1974) and Tsao et al. (1985) that, in guinea pigs, even higher doses of this vitamin can be catabolized very fast without any effect on the total plasma calcium levels.

Awake animals in a sound proof anechoic box in pairs (one control and one AA-treated) were exposed to 1/3 octave band noise centered at 4 kHz. The sound intensity varied near the animal's head (ears) between 113 and 118 dB SPL. Each subject was placed in a body-sized wire cage and located approximately 15 cm in front of the speaker system. The intensity levels were measured through a Brüel and Kjaer microphone No 4134. The exposure period was 2 h.

To minimize the excessive dietary ascorbic acid, all animals were given the standard laboratory chow "MOK" (with an amount of AA not exceeding 2 mg per 1 kg of the diet) 7 days prior to noise exposure and during the surviving period. All experimental animals exhibited no signs of vitamin C deficiency (i.e. reduced diet intake, weight loss, anemia, widespread haemorrhages, etc.).

After surviving for 50 days the animals were killed, the temporal bones were isolated and fixed in 10% neutral formaldehyde solution. The bony cochlear wall was opened from the apex, the basilar membrane (BM) with the organ of Corti was stained in situ by toluidine blue and Ehrlich's haematoxylin and removed from the cochlea coil after coil under a dissecting microscope. Separated

coils of the BM were mounted in glycerine and observed under a light microscope.

The inner (IHC) and outer hair cells (OHC) as well as phalangeal scars in the reticular lamina representing missing hair cells were counted. In order to compare cochleae of different length, each BM was divided into 10 segments of equal length (each segment represented 10% of the whole BM length). The number of IHC and OHC in corresponding segments of the BM were averaged within each group of animals. In the final evaluation the ratio between the number of hair cells (= scars and nonaffected cells) and the number of missing cells (= only scars) in each averaged segment was expressed as a percentage (Table I) and illustrated graphically (Fig. 1). Differences between both groups were tested by a Rank-sum test (Dixon and Massey, 1969). The "SAPI-1" personal computer was used for statistical calculations.

In animals of both groups there was numerical loss of both IHC and OHC along the organ of Corti. The total loss of IHC was higher in control animals (0.34%) than in the AA-treated guinea pigs (0.23%) although the difference was not statistically significant. In none of the segments did the partial loss exceed 1%. There was variation between animals within one group as well as within both cochleae of the same individual.

There was also some variability in losses of OHC; nevertheless, several regularly occurring features can be stated. The maximum loss of OHC in both groups was found in the apical regions (12.57% (control) and 13.9% (AA-treated); Rank-sum test $P = 0.274$). Generally, the loss of OHC became lower towards the base and the difference between both groups became more prominent, the

TABLE I

MEAN VALUES AND STANDARD DEVIATIONS OF OUTER HAIR CELL LOSS IN ASCORBIC ACID (AA) TREATED AND CONTROL (C) GROUP OF GUINEA PIGS.

| Segment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Total | N _{A/E} |
|----------|-------|------|------|------|------|------|------|------|------|------|-------|------------------|
| %OHC(AA) | 13.90 | 3.72 | 3.20 | 0.87 | 0.91 | 0.86 | 0.76 | 1.06 | 0.84 | 0.94 | 2.57 | 5/10 |
| SD(±) | 3.59 | 3.68 | 2.77 | 0.77 | 0.34 | 0.76 | 0.45 | 0.28 | 0.77 | 1.34 | 0.63 | |
| %OHC(C) | 12.57 | 6.78 | 5.73 | 2.44 | 1.29 | 2.40 | 1.42 | 0.87 | 0.69 | 0.69 | 3.58 | 5/9 |
| SD(±) | 2.86 | 9.47 | 6.28 | 2.97 | 0.70 | 1.34 | 0.90 | 0.78 | 0.52 | 0.53 | 1.91 | |

OHC = outer hair cells, SD(±) = standard deviation, N_{A/E} = number of animals/ears examined.

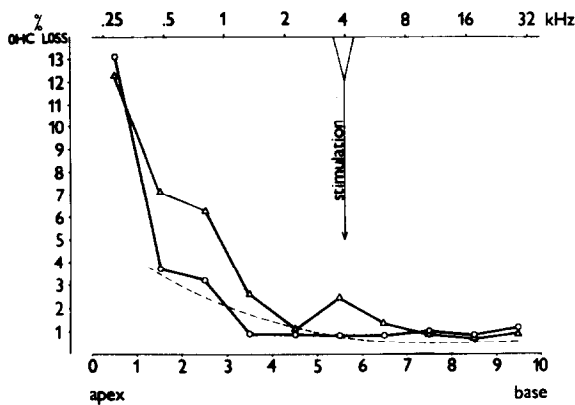


Fig. 1. Graph demonstrating the percentage of the OHC loss in ten segments of the basilar membrane from the apex to base. ○—○ AA-treated group, △—△ nontreated (control) group, dashed line = hair cell loss for 4-month old healthy guinea pigs (according to Úlehlová, 1975).

difference culminating in the course of the sixth segment which corresponds to the region of the basilar membrane where the frequency of 4 kHz used in the experiment should be perceived in guinea pigs (Burda, 1984). Towards the base, the number of missing OHC became again more or less equal in both groups (see Table I, and Fig. 1).

The total loss of OHC in the organ of Corti is 3.58% and 2.57% in nontreated and AA-treated animals, respectively, the difference between these two means being, however, statistically insignificant (Rs-test, $P = 0.21$). Neither the apparent differences between both groups within the second, third and the fourth nor within the seventh segment proved to be statistically significant (Rs-test, $P > 0.5$; $P = 0.5$; $P > 0.5$; and $P > 0.5$, respectively). The difference between both groups in the sixth (i.e. 4 kHz) segment was, however, statistically significant (Rs-test, $P = 0.028$).

Úlehlová (1975) described the distribution of normal, natural loss in numbers of hair cells due to aging. She stated that 0.73% of OHC loss is the norm for 6-week old guinea pigs and 0.85% of OHC loss for 7-month old animals. This value is comparatively much lower even than the total average found in our AA-treated group of guinea pigs which were 4-month old at the end of the experiment. It has to be taken into account, however, that she did not examine the most apical

regions of the organ of Corti, where we ascertained – commonly in the ears of all animals examined – the highest OHC loss. The numerical OHC loss found in AA-treated animals within the sixth segment corresponds to the values given for corresponding cochlear regions in normal 4-month old guinea pigs by Úlehlová (1975) (see dashed line in Fig. 1). It means, that within the 4 kHz frequency segment of the basilar membrane, the hair cell loss after noise exposure has not changed significantly from the norm when animals were treated with ascorbic acid.

As mentioned previously, some investigators have claimed that vitamin A reverses sensorineural hearing loss (Anderson et al., 1950; Baron, 1951). Prophylactic effect was also found in supplemental doses of ATP (c.f. Ebihara, 1963), however, others have demonstrated that vitamin A does not improve sensorineural hearing loss (Ward and Glorig, 1960) and similarly does not protect against noise-induced hearing loss (Ivstam, 1960). Finally, Chole and Quick (1976) found no pathological changes of the neuroepithelium (cochlear and vestibular) in experimental hypovitaminosis A.

A controversy still exists concerning the possible protective or prophylactic effect of some drugs or nutritional compounds against cochlear hair cell degeneration; however, it is apparent that if it exists, we can expect it from substances involved in cochlear metabolism or closely correlated with biochemical processes employed during the acute (acoustic) stress (Selye, 1976). To say that one of those substances is ascorbic acid needs further investigation. The analysis of AA effect on cochlear blood flow, evaluation of the AA concentrations in cochlear tissues, as well as other detailed experiments, seem to be worthwhile. At this point we cannot exclude the possibility that the differences in the hair cell atrophy between both groups of guinea pigs reported here could be caused not only by the protective effect of the drug in AA-treated animals but also partially by the reduction of dietary AA in nontreated individuals (the dietary vitamin C level was very low in this experiment). This interpretation is supported by the work of Cowell (1940), who has found some alterations in various cell types of the cochlea including cells of the organ of Corti after 26-day vitamin C avitaminosis in four guinea pigs.

From the result presented, the following conclusions may be drawn: (1) that some partial prevention of AA against noise induced hair cell degeneration may exist or (2) that even mild hypovitaminosis C potentiates the effect of noise resulting in higher numerical atrophy of cochlear hair cells, (3) a combination of both (1) and (2) is also possible.

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References

- Anderson, J.R., Zoller, H.J. and Alexander, L.W. (1950) Observations in the therapy of deafness and tinnitus with parenteral vitamin A in massive doses. *E.,E.,N.,T. Month.* 24, 75–79.
- Baron, S.H. (1951) Experiences with parenteral vitamin A therapy in deafness and tinnitus. *Laryngoscope* 71, 530–541.
- Burda, H. (1984) Guinea pig cochlear hair cell density; its relation to frequency discrimination. *Hear. Res.* 14, 315–317.
- Chole, R.A. (1980) Autoradiographic localization of vitamin A in the stria vascularis of the rat cochlea. *Arch. Otolaryngol.* 106, 741–743.
- Chole, R.A. and Quick, C.A. (1976) Temporal bone histopathology in experimental hypovitaminosis A. *Laryngoscope* 86, 445–453.
- Cowel, W.P. (1940) Pathologic changes in the peripheral auditory mechanism due to avitaminosis (A, B-complex, C, D, and E). *Laryngoscope* 50, 632–647.
- Darrouzet, J. (1963) Essais de protection de l'organe de Corti contre l'ototoxicité des antibiotiques. *Etude expérimentale. Rev. Laryngol. (Bord.)* 84, 459–478.
- Dixon, W.J. and Massey, F.J.Jr. (1969) Introduction to statistical analysis. Third edn. McGraw-Hill, New York, pp. 638.
- Ebihara, I. (1963) Preventive effect of ATP upon TTS induced by interrupted tone. *Oto-Rhino-Laryng. Clin. (Kyoto)* 56, 326–333.
- Geber, W.F., Anderson, T.A. and Dyne, B. van (1966) Physiologic responses of the albino rat to chronic noise stress. *Arch. Environ. Health* 12, 751–754.
- Ivstam, B. (1960) Vitamin A as a means of reducing industrial deafness. *Acta Otolaryngol.* 52, 321–325.
- Kellerhals, B. (1972) Pathogenesis of inner ear lesions in acute acoustic trauma. *Acta Otolaryngol.* 73, 249–253.
- Pierson, M.G. and Møller, A.R. (1981) Prophylaxis of kanamycin-induced ototoxicity by a radioprotectant. *Hear. Res.* 4, 79–87.
- Selye, H. (1976) Stress in health and disease. Butterworths, 1252 pp.
- Sorenson, D.I., Devine, M.M. and Rivers, J.M. (1974) Catabolism and tissue levels of ascorbic acid following long term massive doses in the guinea pig. *J. Nutr.* 104, 1041–1044.
- Stockwell, C.W., Ades, H.W. and Engström, H. (1969) Patterns of hair cell damage after intense auditory stimulation. *Ann. Otol. Rhinol. Laryngol.* 78, 1144–1168.
- Thorne, P.R. and Gavin, J.B. (1985) Changing relationships between structure and function in the cochlea during recovery from intense sound exposure. *Ann. Otol. Rhinol. Laryngol.* 94, 81–86.
- Tsao, C.S., Young, M., Rose, S.M., Leung, P.Y., Davies, M. Andrews, V. (1985) Effect of ascorbic acid on plasma calcium in guinea pigs. *Internat. J. Vit. Nutr. Res.* 55, 309–314.
- Ukleja, Z. (1967) Protective importance of vitamin A on toxic action of streptomycin on inner ear. *Otolaryngol. Pol.* 21, 715–719.
- Úlehlová, L. (1975) Aging and the loss of auditory neuroepithelium in the guinea pig. In: Cell impairment in aging and development. *Adv. Med. Biol.* 53, 257–264.
- Ward, W.D. and Glorig, A. (1960) The relation between vitamin A and temporary threshold shift. *Acta Otolaryngol.* 52, 72–78.