A Study on the Effects of Sodium Salicylate on the Spiral Ganglion Cells

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살리실산 나트륨이 백서 와우의 나선신경절에 미치는 영향에 대한 연구

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(Received April 21, 1988)

초 록

살리실산이 백서 와우의 나선신경절의 미세구조에 미치는 영향을 전자현미경으로 관찰하였다. 생후 4~5주의 Sprague-Dawley계 백서에 500~600 mg/kg의 용량으로 살리실산 나트륨을 7일간 피하주사한 후에 각각 24시간(1군), 6주(2군), 10주(3군) 경과 후에 와우조직을 얻어 나선신경절에 나타나는 미세구조의 변화를 관찰하였다.

1. 1군에서는 신경절세포와 위성세포 그리고 Schwann cell의 세포질비에 다양한 크기의 막소구의 폭창이 나타났으며 Schwann cell 내에서는 미토콘드리아가 폐손되거나 다양한 세포소체를 형성하였다.

2. 2군에서는 신경절세포와 위성세포내의 막소구들이 더욱 폭창하고 그 일부는 융합하여 세포질에 함몰하였다. Type I 세포의 신경 세포체나 신경섬유 주위를 따고 있는 수초는 각종 사이의 거리가 멀어지거나 일부는 소실되었고, Type II 세포내에는 신경미세유 가 증가하였다.

3. 3군에서는 수초의 변형 및 파괴로 신경절세포의 세포질 이탈이 관찰되었고 Corti기 및 골절 나선판에 위치하는 신경섬유들의 소실이 관찰되었다.

이상의 결과를 종합하면 나선신경절세포와 그 주위를 돌리서는 위성세포 및 Schwann cell은 과형의 살리실산 나트륨 투여후에 미세구조의 변화를 보였으며 1군에서 2군, 3군으로 갈수록 변화가 더욱 현저하였다.

Introduction

The ototoxicity of salicylate has been known for many years. Kirchner, in 1881, found evidence of hemorrhage in the organ of Corti and in the semicircular canals in the human, which
followed after salicylate administration. Thereafter, a few investigators have examined the histological changes in the cochlear structure elicited by salicylate toxicity in the human and experimental animals.

Changes of spiral ganglion cells in the salicylate intoxication were first reported by Wittmaack in 1903. He used guinea pigs and found the disappearance of Nissl bodies of the spiral ganglion cells in experimental salicylate intoxication. Co-vell, in 1936, observed that spiral ganglion cells of guinea pigs, mice, and rabbits showed less amount of ground substance with varying degrees of chromatolysis after salicylate administration. Gotlib (1957) also showed alterations in the spiral ganglion cells of guinea pigs. However, none of these studies showed details of the ultrastructural changes of the spiral ganglion cells sufficiently.

In the present study, I gave high doses of sodium salicylate subcutaneously to rats which have not been previously used in the investigation of salicylate ototoxicity and examined the histological changes of the spiral ganglion cells and nerve fibers using electron microscopy.

Materials and Methods

Three groups of fifteen Sprague-Dawley rats weighing 80~120 gram were injected subcutaneously with a daily dose of 500~600 mg/kg body weight of sodium salicylate in sterile water once a day, for 7 days. Twenty four hours, 6 or 10 weeks after the last injection, animals were killed by decapitation and cochlea were obtained.

Specimens were immersed in 2.5% glutaraldehyde solution buffered with 0.12M sodium phosphate buffer, pH 7.3. The oval and round windows were widened with a dental pick, small holes were made in the apex with a syringe needle followed by perilymphatic perfusion with the same solution. As controls, six animals which were subcutaneously injected with equivalent volume of saline and six normal, healthy, untreated animals of the same age were examined. After the incubation of specimens in 2.5% glutaraldehyde for 3~5 hours at room temperature, specimens were stored overnight at 4°C. They were washed three times in 0.12 M sodium phosphate buffer at pH 7.3 followed by post fixation in 1% OsO₄ in 0.12 M sodium phosphate buffer, pH 7.3, for 2 hours at room temperature. After washing three times in 0.12 M sodium phosphate buffer, pH 7.3, specimens were passed through changes of 30%, 50%, 70%, 80%, 90%, 95% and 100% ethanol (for 10 minutes in each solution). Microdissection was performed in 70% ethanol during dehydration. Specimens were embedded in polybed mixture. Semi-thin sections (1~2μm) were stained with toluidine blue, initially observed with light microscope, and used for orientation for electron microscopy. Trimmed specimens were sectioned with ultramicrotome, stained with uranyl acetate and lead citrate and examined with JEOL 100 CX transmission electron microscope.

Results

At 24 hours survival, varying degrees of distention of membranous cisternae were observed in the cytoplasm of the perikarya of both type I and type II spiral ganglion cells as well as in that of satellite cells (Fig. 8, 9, 12). In the latter, lamellated bodies and formation of myelinated nerve fibers which are folding into the ganglion cell cytoplasm were also seen (Fig. 12). In the myelinated nerve fiber, small vacuoles, myelin figures (Fig. 10, 11) and multicystic cytosomes (Fig. 13) were observed. Unmyelinated nerve fibers showed vacuoles and degeneration of the axon (Fig. 10, 14).

At 6 weeks survival vacuolations became more
extensive and formed large vacuoles or cysts in the ganglion cells or in the satellite cells (Fig. 15, 17, 18). In some of the type II cells, neurofilaments appeared to increase (Fig. 17).

The nerve fibers showed increased interstitial space between the axon and the cytoplasmic process of Schwann cell (Fig. 17, 19), and loosened myelin (Fig. 21) along with the washed appearance of the axoplasm (Fig. 20).

At 10 weeks survival, myelin sheaths around the perikaryon of ganglion cells were broken and the leakage of the cytoplasm of the ganglion cells were observed (Fig. 22). Many concentric lamellated structures were shown in the axoplasm, in or adjacent to the myelin sheath of the myelinated nerve fibers. The cytoplasm of the Schwann cell appeared to be swollen and showed vacuolation (Fig. 25~27). Few normal unmyelinated nerve fibers were observed. Excessive swelling and ruptures of the axonal membranes were observed in the nerve fibers in the osseous spiral lamina and in the organ of Corti (Fig. 23).

Discussion

The present study shows the details of ultrastructural changes in the spiral ganglion cells and nerve fibers caused by the salicylate ototoxicity. These changes appeared in the spiral ganglion cells, satellite cells and the Schwann cells and were more severe in group 2 and 3 animals.

Among these changes, membraneous cisternae were distended to form vacuoles or cysts in the cytoplasm of the ganglion and satellite cells. Schwann cells surrounding axonal process also showed cytoplasmic vacuolations as well as lamellated bodies, enlarged mitochondria and multicystic cytoesomes. Axonal damage including swelling and probably rupture was observed in the nerve fibers present between the sensory cells of the organ of Corti and spiral ganglion. Loosening, folding and rupture of the myelin sheath were also observed around the perikarya and cell processes of the myelinated ganglion cells. Although changes or loss of myelination of spiral ganglion cells have been reported in pathological specimens (Spoendlin, 1974; Ota and Kimura, 1980), its exact mechanism is not known. The abundance of ribosomes in the cytoplasm of the myelinated spiral ganglion cells, satellite cells and Schwann cells indicates an active protein metabolism (Spoendlin, 1966). Therefore, it is possible that above changes of the myelin sheath are caused by the disturbed protein metabolism in salicylate intoxication due to the changes occurred to the membranous cisternae and subsequently to the ribosomes in the perikarya of these cells. However, further investigation of the changes in the more central portion of the vestibulocochlear nerve is required.

Retrograde changes of the peripheral neurons involving the spiral ganglion cells following the cochlear sensory hair cell damage have been reported in mechanical destruction, acoustic trauma (Spoendlin, 1971), intoxication with ototoxic antibiotics (Spoendlin, 1975) or in aging (Anniko, 1985). During the process of retrograde degeneration, loss of myelin sheath and increased quantity of neurofilaments were observed. It proceeded until most of the neurons degenerate and disappear and only 5~10% of them were spared after 5 months (Spoendlin, 1975).

Considering the above reports and our previous study (Ahn et al., 1988), it was inferred that the changes of the spiral ganglion cells and the nerve fibers shown in the present study were secondary to the damaging effects of salicylate on the sensory hair cells. This idea is also supported by the reports of Evans and Borewe (1982) which showed increased random neural
activity of cochlear fibers in salicylate poisoning of cats. The more severe changes at 6 and 10 weeks’ survival than at 24 hours survival might be explained by the fact that retrograde degeneration takes time to proceed through the osseous spiral lamina to the spiral ganglion (Spoendlin, 1975).

As has been described previously by several investigators (Kellerhals et al., 1967; Ross and Burkel, 1973), two types of ganglion cells are found in the spiral ganglion. In all mammalian species, the larger or type I ganglion cells which consist the majority are known to be exclusively connected to the inner hair cells (Spoendlin, 1979). The small or type II ganglion cells are connected to the outer hair cells. In Spoendlin’s study (1981), type I neurons were more subject to pronounced alterations in acoustic trauma or in intoxication with ototoxic antibiotics. However, in our investigation of salicylate ototoxicity, type II neurons showed more severe changes. The difference between Spoendlin’s study and ours may be due to the behavior of the cochlear neurons which varies considerably according to the type of cochlear damage. Its correlation with the fact that the outer hair cells showed more distinctive changes than the inner hair cells in the salicylate intoxication (Ahn et al., 1988) was also suggested.

**Summary**

The ototoxic effects of salicylate on the ultrastructure of spiral ganglion cells were examined. Sodium salicylate (50–60 mg/kg body weight, once a day for 7 days) were injected subcutaneously to 5–6 week-old fifteen Sprague-Dawley rats. Animals were sacrificed 24 hours (group 1), 6 weeks (group 2) or 10 weeks (group 3) after the last injection.

In group 1 animals, distention of membranous cisternae was found in the cytoplasm of ganglion cells, satellite cells and Schwann cells in which enlargement or multicystic cytosome formation of the mitochondria were shown.

In group 2 animals, membranous cisternae became larger or fused to form larger vacuoles or cysts. Shrinkage of spiral ganglion cell cytoplasm and loosening of myelin sheath were seen.

In group 3 animals, extensive swelling or loss of nerve fibers were shown along with the folding or partial loss of myelin sheath which caused leakage of ganglion cell cytoplasm.

It was concluded that the ototoxicity of salicylate caused the ultrastructural changes of the spiral ganglion cells which became more severe in group 2 and 3 animals. The possibility of retrograde degeneration following the sensory cell changes was suggested.

**References**


Kirchner, W. 1881. Über die einwirkung von chinin und salicylsaure auf das Gehörorgan. Berliner Klinische Wochenschrift. 8, 725–726.


Figure Legends

Figs. 1, 2. Light micrographs of the cochlea from the control rat.

Fig. 1. A part of cochlea showing stria vascularis (SV), vestibular membrane (VM), tectorial membrane (T), Corti’s tunnel (CT), vestibular lip or spiral limbus (VL), tympanic lip (TL), habenula perforata (HP), basilar membrane (BM), osseous spiral lamina (OL) and spiral ganglia (SG). ×100.

Fig. 2. Spiral ganglion cells. The majority of them are larger, type I (I) neurons with myelin sheath. Some are smaller type II cells (II). Intraganglionic spiral bundle (IGSB) is seen. S: satellite cell nucleus. ×1,000.

Figs. 3–7. Electron micrographs of the cochlea from the control rat.

Fig. 3. Type I spiral ganglion cell with a surrounding satellite cell. Myelin sheath (M), abundant ribosomes (R) are seen. No: nucleolus of type I ganglion cell, R: ribosome, m: mitochondria, N: nucleus of satellite cell, G: Golgi apparatus, arrows: lysosomes. ×9,000.

Fig. 4. Transverse section through the intraspiral ganglionic bundle showing myelinated (MF) and unmyelinated (unf) nerve fibers. N: nucleus of Schwann cell. ×7,600.

Fig. 5. Type II spiral ganglion cell and the surrounding satellite cell. Note the smaller mitochondria (m) and neurofilament-rich cytoplasmic matrix. No myelin sheath is formed. Arrow: nucleolus of type II ganglion cell, N: nucleus of Schwann cell, m: mitochondria. ×10,000.

Fig. 6. Transverse section through two myelinated nerve fibers in the osseous spiral lamina. M: myelin sheath, A: axon, Arrow heads: inner mesaxon, Arrows: endoneurium, Asterisk: inner cytoplasmic leaflet of Schwann cell. ×50,000.

Fig. 7. Section through the demyelinization zone and inner hair cells (IHC). Neural elements around habenula perforata are seen. HP: habenula perforata, IC: internal pillar cells, CT: Corti’s
tunnel, IP: internal pharyngeal cell. ×3,700.

Figs. 8–13. Electron micrographs of group 1 animals.

Fig. 8, 9. A part of the spiral ganglion. Distended membranous cisternae are seen in the type I(1) and type II(II) ganglion cells, satellite cells (arrows) and Schwann cells (arrow heads). Some of the cisternae appear to be fused (asterisk). N: nucleus of Schwann cell, A: axon, ×4,000(Fig. 8A), ×4,300(Fig. 8B), ×9,300(Fig. 9).

Fig. 10. Nerve filters in the tympanic lip (TL) of the osseous spiral lamina. Note the change of the myelinated nerve fiber (asterisk) and the vacuolation in the unmyelinated nerve fiber (arrow). HA: habenula perforata, IP: internal pharyngeal cell. ×4,000.

Fig. 11. Higher magnification of the myelinated fiber in the osseous spiral lamina which is showing the myelin-like structure enveloped bodies (arrows). ×10,000.

Fig. 12. A type II neuron and the surrounding satellite cell. Note the vacuoles, lamellated bodies (white arrows) and degenerating nucleus (white arrow head) in the satellite cell. Myelin-like annular bands (black arrows) are seen with the inner layers folding into the cytoplasm of type II neuron. ×11,000.

Fig. 13. An myelinated nerve fiber. A multicystic cytosome (asterisk) is seen in the inner cytoplasmic leaflet of the Schwann cell. A: axon, m: mitochondria in axon, M: myelin sheath. ×16,000.

Fig. 14. A part of unmyelinated nerve fiber. Axon (asterisk) is seen being degenerated. ×16,000.

Figs. 15–21. Electron micrographs of group 2 animals.

Fig. 15. A part of spiral ganglion. Ganglion cells are seen with distended membranous cisternae, cavity-like spaces (asterisk) and shrinkage of cytoplasm. Note the partial loss of myelin sheath (arrows) and folding of myelin sheath (arrow heads). I: type I ganglion cell, II: type II ganglion cell. ×2,600.

Fig. 16. A myelinated nerve fiber. Note the enlarged mitochondria (asterisk) in the outer cytoplasmic leaflet of Schwann cell. A: axon, m: mitochondria of Schwann cell, M: myelin sheath. ×22,500.

Fig. 17. A part of spiral ganglion. Note the vacuoles (asterisks) and increased amount of neurofilaments (F) in the ganglion cell. A nerve fiber with increased interstitial space between the cytoplasmic process of the Schwann cell and axon is seen (arrow). ×7,400.

Fig. 18. Type I spiral ganglion cell showing a membrane bounded cyst (asterisk). ×5,200.

Fig. 19. Higher magnification electron micrograph showing the neve fiber with increased interstitial space (arrows). A: axon. ×37,000.

Fig. 20. A myelinated nerve fiber is seen with vacuoles (arrow heads) in the Schwann cell cytoplasm. Axon (A) is showing a partial loss of axoplasm (asterisk). M: myelin sheath. ×35,000.

Fig. 21. A nerve fiber showing a loosening of myelin sheath (M). ×40,000.

Figs. 22–27. Electron micrographs of group 3 animals.

Fig. 22. Spiral ganglion cells. Note that a part of the myelin sheath is broken (arrows) and the cytoplasm is leaking. Empty space (asterisk) after leakage of the cytoplasm. ×3,600.