

The Effects of Sodium Salicylate on the Cochlea of Young Rats; A Structural Study

Young Min Ahn, Byung Lan Lee* and Hyung Ro Moon**

Department of Pediatrics, Kangnam General Hospital Public Corporation, and Department of Anatomy*
and Pediatrics**, College of Medicine, Seoul National University, Seoul 110-744, Korea

= Abstract = The ototoxicity of salicylate has been known and studied for many years. However, there are many discrepancies about the site and mechanism of ototoxic effects of salicylate on the cochlear structure. In order to investigate the ultrastructural changes of the cochlea, high doses of sodium salicylate (500-600 mg/Kg, once a day for 5-7 days) were injected subcutaneously to Sprague-Dawley rats weighing 80-120 gm. The organ of Corti and the stria vascularis were examined under scanning and transmission electron microscopes. Extensive vacuolization which forms characteristic blisters of the cell surface was observed in the outer hair cells. Both in the outer and inner hair cells disarrangement of the stereocilia was seen. It appeared that the apical portions of the cochlea showed more severe changes of stereocilia than basal portions. The stria vascularis did not reveal any distinctive changes.

It was concluded that the ototoxicity of salicylate caused the ultrastructural changes of hair cells in the organ of Corti of rat cochlea and that outer hair cells were more susceptible than the inner hair cells.

Key words: *Salicylate ototoxicity, Rat cochlea, Electron microscopy*

INTRODUCTION

Aspirin has been a popular therapeutic medication because of its freedom from the properties of addiction. However, evidence of salicylate toxicity has been reported, ranging from gastric intolerance to death.

Clinical studies indicated that salicylate toxicity caused an alteration in cochlear function and brought about symptoms such as deafness and tinnitus. Audiometric studies indicated that the locus of aspirin toxicity was intracochlear (McCabe and Dey 1965).

Kirchner, in 1881, found evidence of hemorrhage in the organ of Corti and the semicircular canals in humans, which followed after salicylate administration. Thereafter, a few investigators have

used experimental animals and examined the histological changes in the cochlear structure elicited by salicylate toxicity. Wittmaack (1903) found disappearance of Nissl bodies in the spiral ganglion cells of guinea pigs following experimental salicylate intoxication. Light microscope studies of the inner ear of guinea pigs, mice and rabbits by Covell (1936 & 1938) revealed stria vascular distension and mitochondrial changes in the stria vascularis, and in the outer hair cells. He also showed degenerative changes in the spiral ganglion cells. Falbe-Hansen (1941), with guinea pigs, reported degenerative changes in outer hair cells. In contrast, Gotlib (1957) failed to find any stria or spiral organ abnormalities in guinea pigs after similar treatment, although he reported alterations in the spiral ganglion. Silverstein *et al.* (1967) demonstrated that salicylate does accumulate in the stria vascularis in high concentration.

In more recent studies, Myers and Bernstein (1965) and Deer and Hunter-Duvar (1982), using

squirrel monkeys and chinchillas respectively, failed to find any significant light or electron microscopic changes in the cochlear structure in comparison with control animals. However, Douek *et al.* (1983), using electron microscopy, found that salicylate overdosage caused extensive vacuolation of the smooth endoplasmic reticulum in the periphery of the hair cells, more marked in the outer than in the inner hair cells. Under the scanning electron microscope, the stereocilia of the outer hair cells of the apical two turns became flaccid. No change was found in the stria vascularis.

The results are conflicting and there has been no general agreement on the site and mechanism of action of salicylate in the cochlear structures up to the present time. In the present study, very high doses of sodium salicylate were injected subcutaneously, for several days, to young rats which have not been previously used in the investigation of salicylate ototoxicity and changes in the ultrastructure of the cochlear sensory hair cells and the stria vascularis were examined.

MATERIALS AND METHODS

Nine Sprague-Dawley rats weighing 80-120 gm were injected subcutaneously with a daily dose of 500-600 mg/Kg body weight of sodium salicylate in sterile water once a day, for 5 to 7 days. In the present study, young animals were used in order to compare our results to the ultrastructural changes in Reye's syndrome. Twenty four hours after the last injection, animals were killed by decapitation and cochleas were obtained.

Specimens were immersed in 2.5% glutaraldehyde solution buffered with 0.12 M 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 postfixation 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 dehydrated 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.

For scanning electron microscopy, Reissner's membrane, stria vascularis and tectorial membrane of the organ of Corti were removed and specimens were freeze-dried, gold coated and examined under JSM-T300 scanning electron microscope.

For transmission electron microscopy, specimens were embedded in resin mixture, sectioned and stained in uranyl acetate and lead citrate.

RESULTS

Scanning E. M.

Surface view of the normal spiral organ of Corti showed similar appearance from that of other species (Fig. 1). Findings in the salicylate treated rats varied in minor ways one specimen to another. However, in general, alterations of the stereocilia of outer hair cells and, in severely affected cases, also those of inner hair cells were seen.

In outer hair cells, the stereocilia showed bending (Fig. 2), clumping (Fig. 3, 4) and partial or total loss from the hair cell surface (Figs. 2, 3) were observed. In the severely affected cases, inner hair cells also showed bending and clumping, of the adjacent stereocilia (Fig. 6). Although the severity and localization of the degeneration of the stereocilia varied within each specimen, those of outer hair cells, especially the outermost row, seemed to be most susceptible to the ototoxic effects. In the basal portions of the specimens, the stereocilia of the outer hair cells showed configuration close to normal (Fig. 5).

Transmission E. M.

Hair cells of organ of Corti

Outer hair cells showed marked changes. Varying degrees of vacuolization which formed characteristic blisters of the cell surface was observed (Fig. 8, 9). In the subcuticular area, lysosomes and Hensen's bodies were found more frequently than in control (Figs. 10, 12). Disarrangement of stereocilia, as shown by scanning microscopy, was confirmed (Figs. 10, 13).

In the inner hair cells, similar changes were observed but much less than in outer hair cells (Figs. 15, 16). In addition to disordered stereocilia,

autophagosomes were occasionally seen (Fig. 14).

Stria vascularis

A few vacuoles in variable sizes were shown in the cytoplasm of marginal cells, which were also shown in saline-treated animals (Figs. 17, 18).

DISCUSSION

This report shows that high dosage of salicylate causes the ultrastructural changes in the hair cells of the organ of Corti of young rats.

Both the scanning and transmission electron microscopic examination of the organ of Corti showed that stereocilia of hair cells were vulnerable to salicylate ototoxicity; Bending, clumping and missing of stereocilia were observed. Kohonen, in 1969, suggested that it resulted from the softening of the cuticular plate. However, since the hairs are normally stiff, bending and disarray of hairs were considered to result from a loss of their rigidity (Spondlin, 1960). It may follow the change in fluid balance which causes a loss of turgor in the internal ear (Ross, 1974). This may explain why the stereocilia of the outermost row of outer hair cells, which are the longest of the three rows, appeared to be most prone to bending.

As stated in results, disarray of the stereocilia of hair cells was minimal in the basal portions of the cochlea. It agrees with the results of Douek's histological study. However, it disagrees with those of McCabe and Deys' functional study (1965) which reported that higher frequencies was more affected than the lower. As suggested by Brown *et al.* in 1985, we may conclude that the correlation between the effect of ototoxic agent in the functional study and its effect on the cochlear morphology is not reliable.

Severe vacuolation and bilistering were seen in outer hair cells whereas inner hair cells appeared less affected. This is in agreement with the Douek's findings (1983) in guinea pigs. However, extensive vacuolation of the lateral smooth endoplasmic reticulum which appeared in guinea pigs as the long ribbons of slit-like membrane-lined spaces was not observed in our study. This may result from the different arrangement of endoplasmic reticulum in the normal outer hair cells of the two species. In guinea pigs, several layers of membranous cisternae exist along the plasma membrane while only one layer is observed in rats.

Like the effects of ototoxic aminoglycosides and noise, the present study showed that salicylate affected selectively outer hair cells more than inner hair cells. It is supported by the reports of Silverstein *et al.* (1967) and McCabe and Deye (1965) who suggested that the outer hair cells are a likely site of changes responsible for the hearing loss which occurs after salicylate administration.

Whether the ototoxic effects of salicylate are a direct action of salicylate on the hair cells or an indirect one via the endolymphatic environment is not clearly known. Stria vascularis, which produces endolymphatic fluid, was considered to be involved in the pathological changes of the cochlea in the salicylism by Covell (1936), Myers and Bernstein (1965) and Silverstein *et al.* (1967). However, Anniko (1985) suggested that the toxicity of the drugs was more related to its tissue binding capacity and saturation of receptor sites than to the concentration of the drug in endo- or perilymph. In our investigation, the stria was not altered to any great extent and it supports the Anniko's idea.

Recent studies of Reye's syndrome patients have suggested aspirin treatment as a possible factor on the etiology of this often fatal childhood disorder. However, in the study performed by Rarey *et al.*, in 1984, the ferret model for Reye's syndrome showed extensive vacuolations in the inner hair cells of organ of Corti, stria vascularis, Reissner's membrane, and vestibular sensory epithelium. No vacuoles were seen in the outer hair cells. Since the ferret model for Reye's syndrome were induced by influenza B virus, aspirin and hyperammonemia, we think that differences in the results of Rarey's study and ours are hardly caused by the species difference although different animal species were used. Rather, we may speculate that different pathophysiologicals are involved in the inner ear changes brought about by Reye's syndrome and by aspirine toxicity alone.

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= 국문초록 =

살리실산 나트륨이 白鼠 蝸牛의 微細構造에 미치는 영향에 관한 研究

지방공사 江南病院 小兒科, 서울大學校 醫科大學 解剖學教室* 및 小兒科學教室**

安永敏 · 李秉蘭* · 文炯魯**

Salicylate의 耳毒性은 오래전부터 알려져 왔지만 現在까지 보고된 연구들을 살펴볼 때 salicylate가 蝸牛에 미치는 영향에 관해서는 一致된 見解가 없었다. 따라서 本 實驗은 salicylate投與로 惹起되는 白鼠 內耳에서의 蝸牛의 細胞學的 變化를 면밀히 觀察하고자 施行하였다. 過量의 sodium salicylate (500-600 mg/kg)를 어린 (生後 4-5週) Sprague-Dawley 白鼠에게 5-7日間 매일 投與한 후 走査 및 透過電子顯微鏡(scanning- and transmission electron microscope)을 사용하여 蝸牛의 微細構造 變化를 觀察하여 다음과 같은 結果를 얻었다.

1. 走査電子顯微鏡 觀察時 Corti器의 有毛細胞(hair cell)의 stereocilia가 凝集, 屈曲, 脫落 등의 變形을 보였다. 이와같이 變形은 內有毛細胞에서 보다 外有毛細胞에서 더 심하여 外有毛細胞가 salicylate의 毒性에 더 敏感하다는 것을 알 수 있었고 基部에서 尖端部쪽으로 갈수록 더욱 심한 것으로 觀察되었다.

3. 透過電子顯微鏡 下에서는 stereocillia 配列異狀이 확인되었고 外有毛細胞에는 심한 空胞化현상이 觀察되었으나 內有毛細胞에서는 이와같은 變化가 미미하였다.

3. 血管條는 對照群과 比較하여 뚜렷한 變化를 보이지 않았다.

LEGENDS FOR FIGURES

Figs. 1-6. Scanning electron micrographs

- Fig. 1.** Surface view of organ of Corti in a normal control group. Three rows of outer hair cells(OHC) and one row of inner hair cells(IHC) are seen. The stereocilia of inner hair cells are showing a nearly straight row, and those of outer hair cells are smaller and forming a V or W shape. IP, Head plate of inner pillar cells. Bar = 10 μ m.
- Fig. 2.** Stereocilia of an outer hair cell(asterisk) in a treated rat are wilted and bent over to touch the reticular membrane. Also seen is one outer hair cell with stereocilia part of which are damaged(triple arrows). Bar = 10 μ m.
- Fig. 3.** The outer hair cells in the outermost (OR) and middle (MR) row in a treated rat are showing clumped stereocilia part of which are missing(arrows). IR, innermost row of outer hair cells. Bar = 10 μ m.
- Fig. 4.** The outer hair cells of a treated rat. Clumping of stereocilia is most severe in the outermost row(OR). Some of the stereocilia of one outer hair cell are extending into those of the adjacent outer hair cell(arrow). MR, middle row; IR, innermost row. Bar = 10 μ m.
- Fig. 5.** Outer hair cells in the basal turn in a treated rat. Stereocilia are showing a configuration close to normal except those in the outermost row(OR). Bar = 10 μ m.
- Fig. 6.** Inner hair cells(IHC) in a treated rat showing clumping of stereocilia. OHC, outer hair cells. Bar = 10 μ m.

Figs. 7-18. Transmission electron micrographs

- Fig. 7.** Outer hair cells in a normal control rat. Stereocilia of each cell are consisting of three rows of hairs. D, Dieters cell. x3,300.
- Fig. 8, 9.** outer hair cells of a treated specimen. Note the extensive vacuolations and blistering(arrows) located near the cell surface. x3,000(Fig. 8), x14,000(Fig. 9).
- Fig. 10.** Stereocilia of an outer hair cell in the outermost row of a treated animal is showing bent stereocilia and Hensen's body(HB). x6,000.
- Fig. 11.** Subcuticular area in an outer hair cell of a normal rat is seen. x30,000.
- Fig. 12.** Subcuticular area in an outer hair cell of a treated rat is seen with more lysosomes(asterisks) than in normal. x23,700.
- Fig. 13.** Outer hair cells of a treated animal. Note the stereocilia in a disarrayed configuration. x3,800.
- Fig. 14.** An autophagosome(arrow) is seen in the inner hair cell of a treated animal. x19,000.
- Fig. 15.** Inner hair cell of a normal rat showing well arranged stereocilia. x7,200.
- Fig. 16.** Inner hair cell of a treated animal. Note the disarrangement of stereocilia. x8,500.
- Fig. 17.** Stria vascularis of a control animal. M, Marginal cell; I, intermediate cell; B, Basal cell. x3,800.
- Fig. 18.** Stria vascularis of a treated animal. Almost same or slightly more vacuoles are seen in the cytoplasm of marginal cells(M). I, intermediate cell; B, Basal cell. x4,700.





