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Histopathological Effects of Parylene C (poly-chloro-p-xylylene) in the Inner Ear

Parylene-C'nin (poly-chloro-p-xylylene) İç Kulaktaki Histopatolojik Etkileri

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Original Investigation
Özgün Araştırma

Abstract

Objective: To assess the histopathological effects of parylene C (PC) (poly-chloro-p-xylylene) in the inner ear.

Methods: Nine adult Dunkin Hartley guinea pigs (500–600 g) were included in the study. PC pieces were inserted into the cochlea in the right ear of the animals (study group). The round windows were punctured in the left ears comprised the control group. After three months, the animals were sacrificed, and the dissected temporal bones were examined under a light microscope.

Results: No significant difference was revealed between the study and control groups regarding histopathological findings such as perineural congestion, perineural inflammation, neural fibrosis, number of ganglion cells, edema, and degeneration of ganglion cells (p>0.05).

Conclusion: PC did not cause any additional histopathologic damage in the cochlea. This finding may be promising regarding the use of PC in cochlear implant electrodes as an alternative to silicon materials in the future.

Keywords: Cochlear implant, parylene C, silicone

Öz

Amaç: Parilen C (PC) (poly-chloro-p-xylylene)'nin iç kulaktaki histopatolojik etkilerini değerlendirmek.

Yöntemler: Dokuz adet erişkin Dunkin Hartley kobayı (500-600 gram) çalışmaya dahil edildi. PC parçaları kobayların sağ kokleasına yerleştirildi (çalışma grubu). Sol taraf yuvarlak pencereleri kontrol grubunu oluşturmak amacıyla rüptüre edildi. Üç ay sonrası hayvanlar sakrifiye edildi ve diseke edilen temporal kemikleri ışık mikroskopu altında incelendi.

Bulgular: PC ve kontrol grubu arasında perinöral konjesyon, perinöral enflamasyon, nöral fibröz, ganglion hücre sayısı, ganglion hücrelerinde ödem ve dejenerasyon gibi histopatolojik bulgular açısından anlamlı bir fark bulunmadı (p>0.05).

Sonuç: PC'nin kokleada ek bir histopatolojik hasara yol açmadığı gözlendi. Bu bulgular PC'nin ileride silikona alternatif olarak yeni bir implant kaplama materyali olarak kullanımına dair umut vericidir.

Anahtar Kelimeler: Koklear implant, parilen C, silikon

Introduction

Cochlear implants have become extremely successful neural prostheses since their introduction in the 1980s. To date, thousands of people worldwide who have severe hearing loss have received cochlear implants (1). As silicon-based materials have an excellent biocompatibility, they are being used in the fabrication of a wide range of biomedical devices for diagnostic and therapeutic applications such as in neural electrodes and implantable sensors (2). They have been used for many years in cochlear implantation. However, there are some disadvantages of silicon-based cochlear implants. These implants are relatively brittle and stiff, which may cause a break during insertion (3). In addition, failures of silicon-based cochlear implants due to inflammatory and allergic reactions have been reported (4-9).

Parylene technology has been used in various medical applications. The most common parylene materials are parylene N, parylene D, and parylene C [poly(chloro-p-xylylene)] (PC). PC is hydrophobic and is conformably deposited and chemically inert toward most common organic and biological fluids and water till up to 150˚C (10). It has a high

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resistance and elasticity, low dielectric constant, and the highest biocompatibility certification with United States Pharmacopeial Class VI (11, 12).

In this study, we aimed to investigate the histopathological effects of PC in the inner ear.

Methods

Nine adult Dunkin Hartley guinea pigs (500–600 g) were included in the study. Approval for the study was obtained from the local animal ethics committee. The right and left ears of the same animal comprised the study and control groups, respectively.

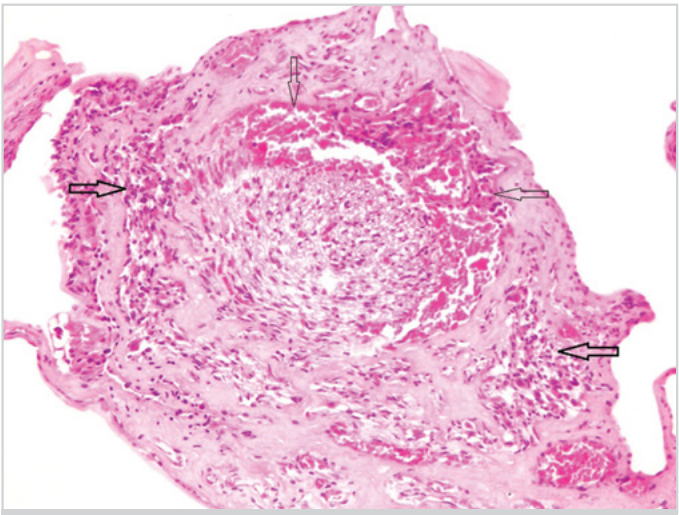

Figure 1. Perineural congestion (thin arrow) and perineural inflammation (thick arrow) shown in the nerve section (H&E, 200×)

Surgical procedure: General anesthesia for the guinea pigs was achieved with 5 mL ketamine HCI (Ketalar®; Eczacıbaşı Warner Lambert, İstanbul, Turkey), and 4 mL xylazine (Rompun®; Bayer Vital, Leverkusen, Germany) was used for muscle paralysis. During the surgical procedure, a Carl Zeiss OPMI 9-FC® microscope (Goettingen, Germany) was used. Tympanic and round window membranes on the right ear (study group) were ruptured via a zero degree needle, and subsequently, 2-mm-long ribbon PC pieces were inserted into the cochlea via the round window with a micro-ear alligator forceps. In the left ear (control group), the tympanic and round window membranes were ruptured in the same way, and no other application was performed. The animals were sacrificed three months later. The temporal bones were removed to perform a histopathological examination.

Histopathological examination: Ribbon PC pieces were removed from the cochlea in the study group. All temporal bones of the guinea pigs were fixed in 10% buffered formalin for 72 hours and subsequently decalcified in formic acid for 2 weeks. Formic acid solution was replaced every other day. After decalcification, the samples were rinsed with running tap water for 1 hour and were placed in a tissue pursuit device. Next day, 3-μ-serial sections were cut from the paraffin-embedded blocks

Table 1. Histopathological findings of the study group and the control group

	Perineural congestion	Perineural inflammation	Neural fibrosis	Edema and degeneration of ganglion cells	Number of ganglion cells/ HPF
C1	+	1	2	2	50.00
C2	+	1	1	2	50.00
C3	+	1	1	2	30.00
C4	+	1	1	CNE	CNE
C5	+	1	1	2	60.00
C6	+	1	0	0	80.00
C7	+	2	2	3	10.00
C8	+	1	1	2	60.00
C9	+	1	1	2	80.00
S1	+	1	0	1	70.00
S2	+	1	0	1	80.00
S3	+	2	1	2	50.00
S4	+	1	1	1	80.00
S5	+	1	1	1	50.00
S6	+	1	1	1	80.00
S7	+	1	1	2	70.00
S8	+	1	1	1	70.00
S9	+	1	1	1	85.00

C: control; S: study; +: present; 0: absent; 1: mild; 2: moderate; 3: severe; CNE: could not be evaluated; HPF: high-power field

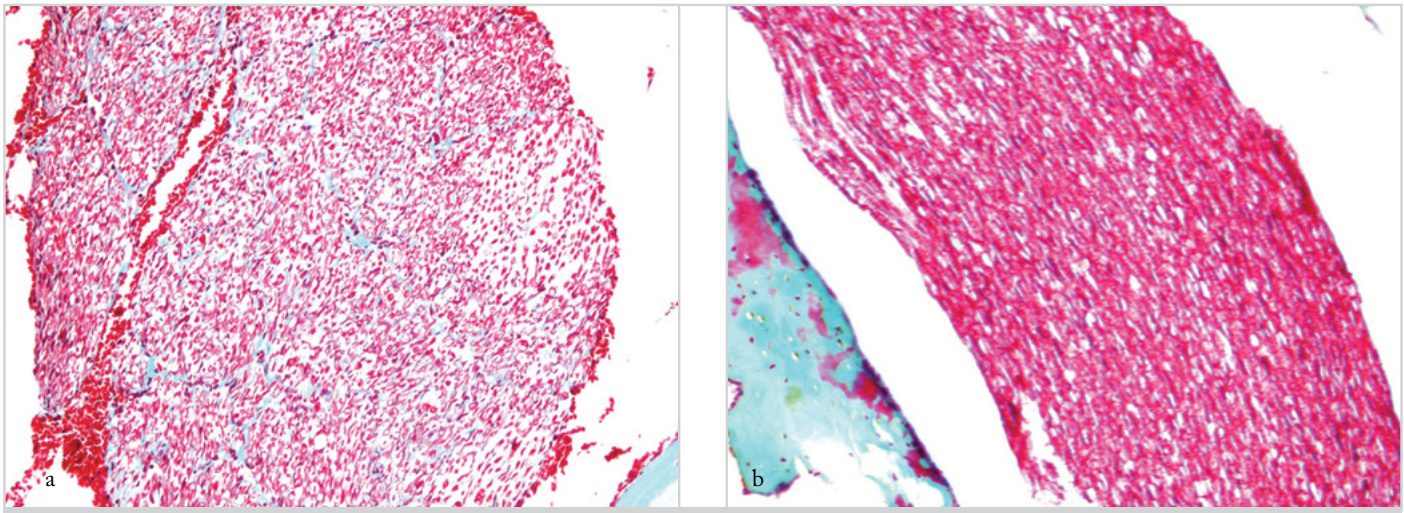

Figure 2. a, b. Severity of fibrosis observed in the nerve section (a) Moderate fibrosis: connective tissue fibers widely stained green with trichrome dye (b) No fibrosis: nerve section lacking green-stained areas (trichrome, 200×)

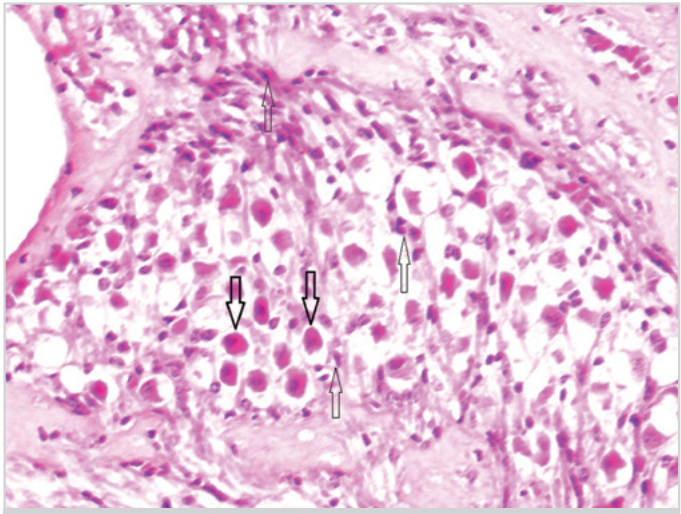

Figure 3. Mild edema and degeneration of ganglion cells. Thick arrow: Normal ganglion cells, thin arrow: degenerated ganglion cells (H&E, 400×)

and stained with hematoxylin–eosin H&E and trichrome dyes. The sections were examined under a light microscope (Olympus BX51; Tokyo, Japan). Histopathologically, five parameters were evaluated: perineural congestion and inflammation, neural fibrosis, number of ganglion cells, edema, and degeneration of ganglion cells.

Perineural congestion was scored as + (present) or – (absent). Perineural inflammation was scored from 0 to 3, with 0 indicating absent, 1 (mild) indicating 25% inflammation, 2 (moderate) indicating 26–50% inflammation, and 3 (severe) indicating >50% inflammation.

Ganglion cell count was calculated under one high magnification (40×) light microscopy after identifying the densest region of ganglion cells. Edema and degeneration of the ganglion cells were scored as 0 indicating no edema or degeneration of the cells, 1 indicating edema and degeneration in up to 25% of the cells, 2 indicating edema and degeneration in 26–50% of the cells, and 3 indicating edema and degeneration in more than 50% of the cells. Trichrome dye was used to evaluate neural fibrosis histochemically. Sparse, thin fibers stained greenish observed in neural sections were scored as 1 (mild fibrosis), whereas denser, thicker fibers were scored as 2 (moderate fibrosis). It was scored as 0 if there was no fiber stained with trichrome dye in the nerve section.

Statistical Analysis

SPSS for Windows 22.0 (IBM Corp; Armonk, New York, USA) was used for statistical analysis. Pearson Chi-Square test was used to compare the histopathological findings. The number of ganglion cells was compared using paired sample t-test; in all tests, $p<0.05$ was considered to be statistically significant.

Results

The histopathological findings of both groups are shown in Table 1. Perineural congestion was observed in all animals in the study and control groups (Figure 1). There was no significant difference between the groups regarding perineural inflammation ($p=0.708$) or neural fibrosis severity ($p=0.526$) (Figure 2). Likewise, no significant difference was revealed between the groups regarding edema and degeneration of the ganglion cells ($p=0.169$) (Figure 3). The mean number of ganglion cells in the study and control groups was 69.3±13.2 and 52.5±23.7, respectively, which was not statistically significant ($p=0.062$).

Discussion

The causes of cochlear implant failure are extrusion and malpositioning of the implant electrode, wound and flap problems, and trauma (13-16). In addition, few authors have mentioned that allergy to silicon is one of the causes of cochlear implant extrusion (7-9). Puri et al. (9) reported contact dermatitis developing after cochlear implantation. In a skin patch test, they diagnosed allergy to silicone LSR-30 found in the device. Shao (17) reported cochlear implant failure secondary to the restoration of silicone during device manufacturing.

PC has been widely used as a coating material for isolating implantable biomedical devices (18, 19). It has a number of fea-

tures such as its high molecular weight, all-carbon structural backbone, and nonpolar entities, which prevents contaminations by most chemicals, fungi and bacteria (20). It has been reported that PC is more hemocompatible and less thrombogenic than silicon and that it has a high stability in vivo for many biological and biomedical applications (21). The biocompatibility of PC has been shown in bladder tissue (22).

The surface characteristics and cell and protein compatibility of PC is comparable with those of polystyrene, polydimethylsiloxane, and glass. PC substrates preserve their hydrophilic properties over time and display a higher degree of nanoscale surface roughness (>20 nm) than other substrates (22). Therefore, PC can be a useful material for fabricating cell-based microdevices (19).

The biocompatibility of polyimide or polyimide coated with amorphous aluminum oxide, amorphous carbon, parylene, polyvinylpyrolidone (PVP), or polyethylene glycol (PEG) was evaluated for possible use in subretinal prostheses. PEG, parylene, and PVP have been shown to produce less histologic disruption than other compounds. In addition, there was no significant difference between parylene, PEG, and the nonsurgical control group in disturbing retinal anatomy (18). In a similar study, polyimide, parylene, and silicone were evaluated as retinal prosthesis electrode array substrate materials. When compared in terms of biocompatibility, PC showed an excellent long-term performance (23).

Parylene has also been assessed as a coater for silicon in cochlear implant electrodes in a previous study. It was proposed that silicon cochlear electrodes would be more flexible and robust after encapsulation with parylene (3).

In our study, the possible effects of PC in the inner ear were examined. No significant difference was revealed between the study and control groups regarding histopathological findings. Compared to the controls, it seems that PC did not cause additional histopathological damage to the cochlea. This finding is promising for the development of a new implantable material coater to overcome silicon-based cochlear implant failures.

Conclusion

In conclusion, PC did not cause any histopathologic damage in the cochlea. This finding may be promising regarding the use of PC in cochlear implant electrodes as an alternative to silicon materials. However, further studies are needed to assess the value of PC as an alternative implant coater.

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