· 实验研究 ·

膦甲酸钠不同眼内注射方式对角膜及视网膜的毒性作用

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【摘要】 **目的**　评估膦甲酸钠前房和玻璃体腔注射对角膜及视网膜的毒性。 **方法**　选取成年新西兰白兔 36 只，采用随机数字表法将其随机分为对照组、玻璃体腔注药组和前房注药组，每组 12 只，其中对照组一侧眼玻璃体腔注射 0.1 ml 平衡盐溶液（BSS），另一侧眼前房内注射等容积 BSS；玻璃体腔注药组和前房注药组分别于单眼玻璃体腔和前房内注射 0.1 ml 膦甲酸钠 1.2 mg。于注射后第 1、7、14、28 天分别对 3 只实验兔进行裂隙灯显微镜、检眼镜、光学相干断层扫描、活体扫描共聚焦显微镜检查，处死后摘取双眼眼球分别对角膜及视网膜行光学显微镜、扫描电子显微镜和透射电子显微镜检查，综合评估膦甲酸钠对角膜和视网膜的毒性。 **结果**　裂隙灯显微镜、光学相干断层扫描结果显示，玻璃体腔注药组和对照组均未见角膜水肿、眼内炎症或其他异常。前房注药组注射后 1 d 可见角膜轻度水肿，注射后 7 d 角膜水肿消退。活体扫描共聚焦显微镜结果显示，玻璃体腔注药组和前房注药组角膜内皮细胞呈典型六边形，未见形态学异常。玻璃体腔注射 BSS 组、前房注射 BSS 组、玻璃体腔注药组和前房注药组注药前和注药后 1、7、14 d ECD 总体比较差异均无统计学意义（$F_{分组}=1.21$，$P=0.32$；$F_{时间}=1.21$，$P=0.32$）。光学显微镜下观察结果显示，各组注药后不同时间点角膜形态学均未见明显异常。玻璃体腔注药组和前房注药组注射后 1 和 7 d 均可见视网膜神经纤维层空泡化，伴大量炎性细胞浸润。玻璃体腔注射 BSS 组注射后 1 d 神经纤维层内也出现炎症细胞浸润，但未见空泡化改变。光感受器层无结构改变，细胞核层组织良好。扫描电子显微镜下观察结果显示，玻璃体腔注药组注射后 1 d 角膜内皮未见明显异常。前房注药组注射后 1 d 可见大量炎性细胞沉积并黏附在角膜内皮上，注射后 7 d 消失。透射电子显微镜下观察结果显示，玻璃体腔注药组注射后 1 d 角膜内皮细胞肿胀，内质网扩张，部分线粒体肿胀，注射后 14 d 恢复正常；注射后 1 d，视网膜神经纤维层可见泡状结构，注射后 28 d 仍有组织间液残留。前房注药组注射后 1 d 可见角膜内皮细胞线粒体和内质网肿胀，14 d 后恢复正常；注射后 1 d 视网膜外节膜盘结构异常，视神经纤维层产生组织间液，至注射后 28 d 未完全恢复。 **结论**　膦甲酸钠前房和玻璃体腔注射对视网膜有短暂毒性作用，随时间延长作用逐渐减弱。膦甲酸钠前房内注药对角膜内皮细胞的毒性作用较玻璃体腔内注药更显著。

【关键词】 膦甲酸钠；玻璃体腔注药；前房注药；药物毒性；角膜；视网膜
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Retinal and corneal toxicity analysis of different intraocular injection methods of foscarnet sodium in rabbit eyes

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[**Abstract**] **Objective**　To evaluate the toxicity of foscarnet sodium injection into the anterior chamber and intravitreal cavity on the cornea and retina. **Methods**　Thirty-six adult New Zealand White rabbits were randomly divided into control group, intravitreal injection group, and intracameral injection group, with 12 rabbits in each group. In the control group, 0.1 ml of balanced salt solution (BSS) was injected into the vitreous cavity of one eye, and an equal volume

of BSS was injected into the anterior chamber of the other eye. In the intracameral injection group and intravitreal injection group, 0.1 ml of sodium foscarnet 1.2 mg was injected into the anterior chamber and vitreous cavity of one eye, respectively. Slit-lamp microscopy, ophthalmoscope, optical coherence tomography (OCT), and *in vivo* confocal laser scanning microscopy were performed on 3 experimental rabbits from each group on days 1, 7, 14, and 28 after injection. After sacrifice, both eyeballs were removed, and the corneas and retinas were examined using optical microscopy, scanning electron microscopy and transmission electron microscopy to evaluate the toxicity to the cornea and retina comprehensively. The use and care of the animals complied with the ARVO Statement. The study protocol was approved by an Ethics Committee of Peking University Third Hospital (No. IRB00006761-2015197). **Results** Slit-lamp microscopy and OCT showed no corneal edema, intraocular inflammation, or other abnormalities in the intravitreal injection and control groups. Mild corneal edema was observed in intracameral injection group 1 day after injection, which resolved 7 days after injection. *In vivo* confocal laser scanning microscopy revealed normal hexagonal corneal endothelial cell morphology in the intravitreal injection and control groups. There was no significant difference in endothelial cell density at baseline and 1, 7, and 14 days after injection among the three groups ($F_{group}=1.21$, $P=0.32$; $F_{time}=1.21$, $P=0.32$). Light microscopy revealed no obvious corneal abnormalities. On days 1 and 7 after injection, retinal nerve fiber layer vacuolization and inflammatory cell infiltration were observed in the intravitreal injection and control groups. In the intravitreal injection of BSS group, inflammatory cell infiltration occurred in the retina without vacuolization 1 day after injection. There were no structural changes in the photoreceptor layer, and the nuclear layer was well-organized. Scanning electron microscopy showed no significant abnormalities in the corneal endothelium in the intravitreal injection group 1 day after injection. In the intracameral injection group, a large number of inflammatory cells were deposited and adhered to the corneal endothelium 1 day after injection and disappeared 7 days after injection. Transmission electron microscopy revealed that in the intravitreal injection group, 1 day after injection swelling of corneal endothelial cells, dilatation of the endoplasmic reticulum, and partial mitochondrial swelling were observed, which normalized 14 days after injection and vacuolization was present in the retina and interstitial fluid accumulation persisted until the 28 days after injection. In the intracameral injection group, swollen mitochondrial and endoplasmic reticulum of corneal endothelial cells was observed and resolved by 14 days after injection. However, structural abnormalities in the membranous discs of the photoreceptor outer segments and interstitial fluid accumulation in the optic nerve fiber layer persisted 1 day after injection and did not fully recover 28 days after injection. **Conclusions** Intracameral intravitreal and injection of foscarnet sodium have transient toxic effects on the retina, which gradually weaken over time. Intracameral injection of foscarnet sodium was more toxic to corneal endothelial cells than intravitreal injection.

[**Key words**] Foscarnet sodium; Intravitreal injections; Intracameral injection; Toxicity; Cornea; Retina

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眼球各个部位均可受到病毒的感染，导致睑缘炎、结膜炎、角膜炎、葡萄膜炎、白内障和视网膜炎在内的各类眼部疾病[1]。眼内病毒感染可导致严重的视力损害，甚至盲[2-5]。因此，有效的抗病毒治疗对于抑制病毒复制、减少组织损伤和提高患者视力十分重要。病毒性角膜炎是一类严重影响视力的眼内感染性疾病。目前，针对病毒性角膜炎的传统治疗方法包括静脉输注更昔洛韦和口服缬更昔洛韦[6]。然而，更昔洛韦耐药性的出现使病毒性角膜炎的治疗复杂化，且常出现于免疫功能异常的患者中，会导致病程延长和并发症发生概率升高[7-9]，因此亟需探索可替代的角膜抗病毒治疗手段。

膦甲酸钠作为治疗抗病毒药物耐药和难治性病毒感染的一线药物，可以通过与 DNA 聚合酶的焦磷酸盐结合位点直接结合发挥抗病毒作用[10-13]。然而，膦甲酸钠静脉输注有严重的药物毒性，尤其是肾毒性，使其全身用药的时长、浓度均受到一定限制[10,13-14]。此外，由于血-眼屏障的存在，导致全身用药后眼内药物浓度低[15]。与全身给药相比，玻璃体腔注射膦甲酸钠可直接将药物送到眼部，有效提高局部药物浓度，减少用药剂量，降低全身用药的毒性作用，更好地控制眼内病毒复制。因此，玻璃体腔注射膦甲酸钠目前已成为

治疗急性视网膜坏死和巨细胞病毒性视网膜炎的有效手段[16-18]。目前，关于玻璃体腔注射膦甲酸钠的安全性评估研究数量较少，尽管已有少量研究报道玻璃体腔注射膦甲酸钠无视网膜毒性[16-18]，但部分研究指出玻璃体腔注射膦甲酸钠后出现了结晶化[19-20]。此外，评估玻璃体腔注射膦甲酸钠对角膜内皮毒性的相关研究仍缺乏。对于难治性病毒性角膜内皮炎，尤其是对于更昔洛韦耐药的病例，膦甲酸钠眼部用药作为一种可能有效的治疗手段，其给药途径和安全性尚未可知。通过前房注药可以直接提高角膜附近药物浓度，从而增强对角膜病毒感染的控制效果。由于人角膜内皮细胞不可再生，较高的药物浓度可能导致药物毒性角膜内皮细胞损伤，因此在临床用药前，需完善膦甲酸钠前房注药的安全性和细胞毒性评估，减少用药风险。本研究旨在评估兔眼球内注射膦甲酸钠（玻璃体腔注射和前房注射）对角膜内皮和视网膜组织的安全性，为膦甲酸钠眼内不同部位注射治疗难治性病毒性角膜内皮炎提供证据支持。

1　材料与方法

1.1　材料

1.1.1　实验动物　选取成年新西兰白兔 36 只，体质量 2.5~3.0 kg，购自北京维通利华实验动物技术有限公司。所有白兔实验前排除眼部感染、炎症等异常，于室温、光线充足的环境饲养，自由饮水进食。所有实验及动物喂养均遵循美国视觉与眼科学研究协会关于使用动物进行研究的决议进行，研究方案经北京大学第三医院伦理委员会批准（批文号：IRB00006761-2015197）。

1.1.2　主要试剂及仪器　膦甲酸钠（连云港市正大天晴药业集团股份有限公司）；平衡盐溶液（balanced salt solution，BSS）（上海国药集团化学试剂有限公司）。光学相干断层扫描仪（美国 Optovue 公司）；活体扫描共聚焦显微镜（德国 Heidelberg 公司）；光学显微镜（日本尼康公司）；扫描电子显微镜（JSM-5600LV）、透射电子显微镜（JEM-1230）（日本电子株式会社）。

1.2　方法

1.2.1　实验动物分组及处理　采用随机数字表法将 36 只实验兔分为对照组、玻璃体腔注药组和前房注药组，每组 12 只，其中对照组一侧眼于玻璃体腔内注射 0.1 ml BSS，另一侧眼前房内注射等容积 BSS；玻璃体腔注药组和前房注药组分别行单眼玻璃体腔和前房内注射 0.1 ml 膦甲酸钠 1.2 mg。

1.2.2　球内注射　注射前均先行前房穿刺术降低眼压，避免药物从穿刺处溢出。行前房注射时，使用 29G 注射器抽吸药物后于角膜缘后 2 mm 行前房注药。药物通过 29G 针头经过角膜缘进入前房。行玻璃体腔注射时，使用 29G 注射器抽吸药物后于角膜缘后 2 mm 处缓慢注射药物，避免损伤晶状体。

1.2.3　眼部形态学检查及角膜相关参数评估　注射前和注射后 1、7、14、28 d，采用裂隙灯显微镜和检眼镜进行眼前节照相以及眼底检查，主要评估角膜水肿、眼内炎症、玻璃体混浊、玻璃体出血、视网膜脱离、视网膜前膜形成和新生血管形成情况。采用光学相干断层扫描仪进行角膜厚度及角膜水肿程度评估；采用活体扫描共聚焦显微镜进行角膜内皮细胞密度（endothelial cell density，ECD）检查。

1.2.4　角膜和视网膜的组织学观察　分别于注射膦甲酸钠后第 1、7、14、28 天静脉注射过量戊巴比妥钠对实验兔实施安乐死，每个时间点每组取 3 只实验兔，并立即摘除眼球，固定角膜及视网膜样本。

1.2.4.1　苏木精-伊红染色观察角膜和视网膜形态学特征　将样本在 4% 甲醛溶液中室温固定 24 h，梯度乙醇脱水（浓度递增），二甲苯中浸泡后石蜡包埋。4.0 μm 厚垂直角膜连续切片后置于载玻片上，二甲苯脱蜡，梯度乙醇水合（浓度递减）。水合后的切片用苏木素溶液染色 10 min，自来水冲洗 15 min，0.5% 伊红溶液染色 10 min，梯度乙醇脱水（浓度递增），二甲苯中浸泡，合成树脂溶液中封片，光学显微镜下观察细胞形态及角膜各层结构。

1.2.4.2　扫描电子显微镜检查　将样本在含 3% 戊二醛的磷酸盐缓冲液（phosphate buffer saline，PBS）中室温固定 2 h，用 PBS 洗涤 3 次，梯度乙醇脱水。样本临界点法干燥后，涂 20 nm 金钯导电涂层，扫描电子显微镜下观察细胞间连接及细胞表面微绒毛结构。

1.2.4.3　透射电子显微镜检查　将样本在 3% 戊二醛中室温固定 1 h，再用 1% 四氧化锇固定 2 h，PBS 洗涤 3 次，每次 5 min，梯度乙醇脱水，包埋于 Epon 812 中。使用铜网收集标本切片（80 nm）后用乙酸双氧铀和柠檬酸铅进行双重染色，透射电子显微镜下观察细胞间连接及细胞器形态变化。

1.3　统计学方法

采用 SPSS 20.0 统计学软件进行统计分析。计量资料数据经 Shapiro-Wilk 检验证实符合正态分布，以 $\bar{x}\pm s$ 表示，各组间不同时间点 ECD 比较采用两因素方差分析。$P<0.05$ 为差异有统计学意义。

2　结果

2.1　各组裂隙灯显微镜、光学相干断层扫描和眼底检查结果比较

玻璃体腔注药组和对照组注射前后均未见角膜水肿、眼内炎症或其他异常(图 1,2)。前房注药组注射后 1 d 角膜轻度水肿,注射后 7 d 角膜水肿消退(图 1);光学相干断层扫描显示注射后 1 d 角膜轻度增厚,注射后 7 d 厚度恢复正常(图 2)。眼底检查均未见异常。

2.2　各组活体扫描共聚焦显微镜检查结果比较

玻璃体腔注药组和前房注药组角膜内皮细胞呈典型六边形,注药后 7、14 d 均未见形态学异常(图 3)。玻璃体腔注射 BSS 组、前房注射 BSS 组、玻璃体腔注药组和前房注药组注药前和注药后 1、7、14 d ECD 总体比较,差异均无统计学意义($F_{分组}=1.21$, $P=0.32$; $F_{时间}=1.21$, $P=0.32$)(表 1)。

2.3　各组光学显微镜检查结果比较

各组注药后不同时间点角膜形态学均未见明显异常(图 4)。玻璃体腔注药组和前房注药组注射后 1 和 7 d 均可见视网膜神经纤维层空泡化,伴大量炎性细胞浸润。前房注射 BSS 组视网膜各层结构未见明显异常,玻璃体腔注射 BSS 组注射后 1 d 神经纤维层内也出现炎症细胞浸润,但未见空泡化改变。光感受器层无结构改变,细胞核层组织结构良好(图 5)。

2.4　各组扫描电子显微镜检查结果比较

扫描电子显微镜下观察结果显示,玻璃体腔注药组注射后 1 d 角膜内皮细胞结构正常,六角形细胞边界清晰,细胞间连接紧密,内皮细胞彼此相互交错,细胞表面微绒毛结构正常;前房注药组注射后 1 d 可见大量炎性细胞沉积并黏附在角膜内皮上,注射后 7 d 消失(图 6)。

2.5　各组透射电子显微镜检查结果比较

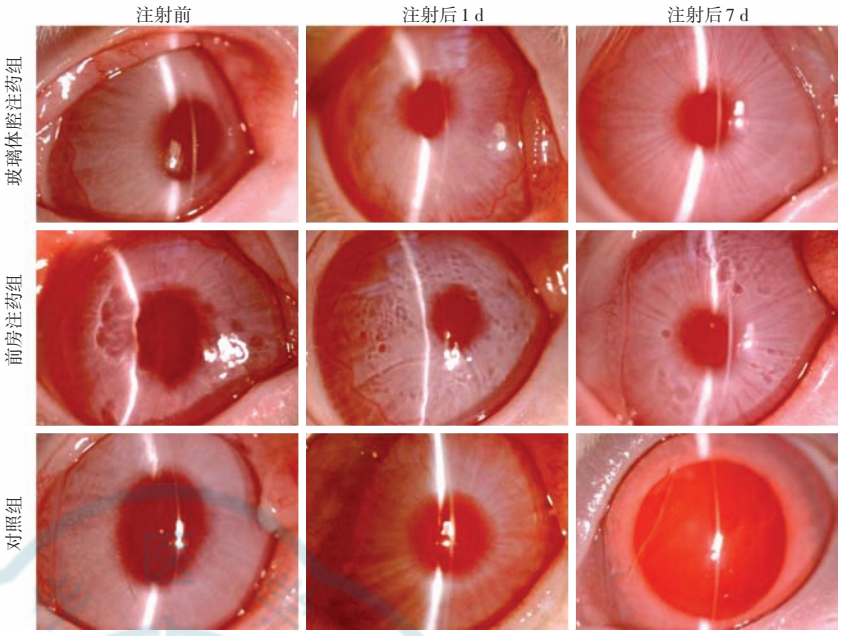

图 1　各组实验兔眼前节照相　玻璃体腔注药组和对照组均未见角膜水肿、眼内炎症或其他异常,前房注药组注射后 1 d 角膜轻度水肿,注射后 7 d 角膜水肿消退

Figure 1　Anterior-segment photographs of rabbit eyes　No corneal edema, intraocular inflammation, or other abnormalities were observed in the intravitreal injection and control groups. Mild corneal edema was observed in intracameral injection group 1 day post-injection, which resolved by 7 days post-injection

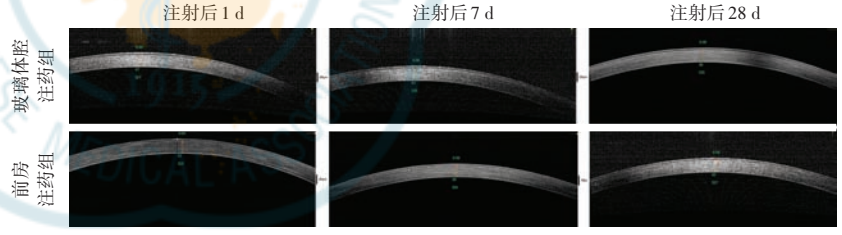

图 2　各组实验兔光学相干断层扫描图像　玻璃体腔注药组注射后不同时间点角膜均未见明显异常,前房注药组注射后 1 d 角膜增厚,注射后 7 d 厚度恢复正常

Figure 2　Optical coherence tomography of rabbit eyes　No significant corneal abnormalities were observed at any time point after injection in the intravitreal injection group. The intracameral injection group showed corneal thickening 1 day post-injection, which returned to normal by 7 days post-injection

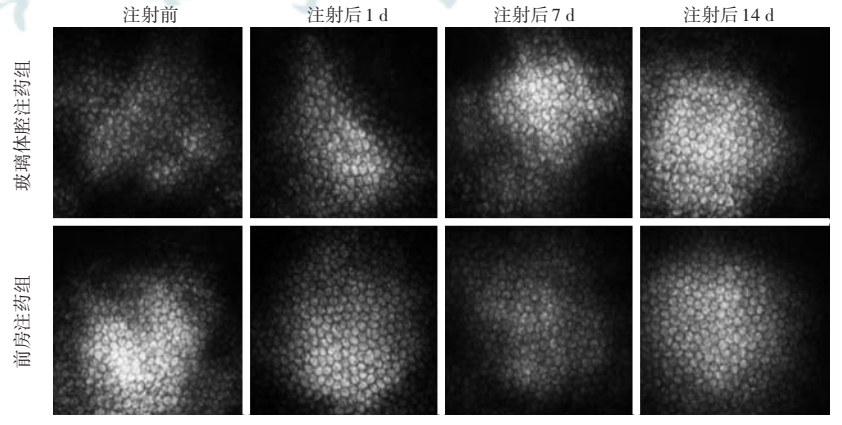

图 3　兔眼角膜内皮细胞层典型活体扫描共聚焦显微镜图像　玻璃体腔注药组和前房注药组角膜内皮细胞呈典型六边形,未见形态学异常

Figure 3　*In vivo* confocal microscopy images of the endothelial layers of rabbit eyes　Corneal endothelial cells in both the intravitreal injection group and the intracameral injection group exhibited typical hexagonal morphology, with no morphological abnormalities

表 1　各组注药前后不同时间点 ECD 比较（$\bar{x}\pm s$，个/mm²）

Table 1　Comparison of ECD before and after injection among different groups（$\bar{x}\pm s$, cells/mm²）

组别	样本量	注射前	注射后 1 d	注射后 7 d	注射后 14 d
玻璃体腔注药组	3	2 775±217	2 725±115	2 725±175	2 742± 80
前房注药组	3	2 767±141	2 708±142	2 708±113	2 542±123
玻璃体腔注射 BSS 组	3	2 667±236	2 625±327	2 600±229	2 583±153
前房注射 BSS 组	3	2 817±126	2 742±126	2 642± 88	2 658± 38

注：$F_{分组}=1.21$，$P=0.32$；$F_{时间}=1.21$，$P=0.32$（两因素方差分析）　ECD：内皮细胞密度；BSS：平衡盐溶液

Note：$F_{group}=1.21$，$P=0.32$；$F_{time}=1.21$，$P=0.32$（Two-way ANOVA）　ECD：endothelial cell density；BSS：balanced salt solution

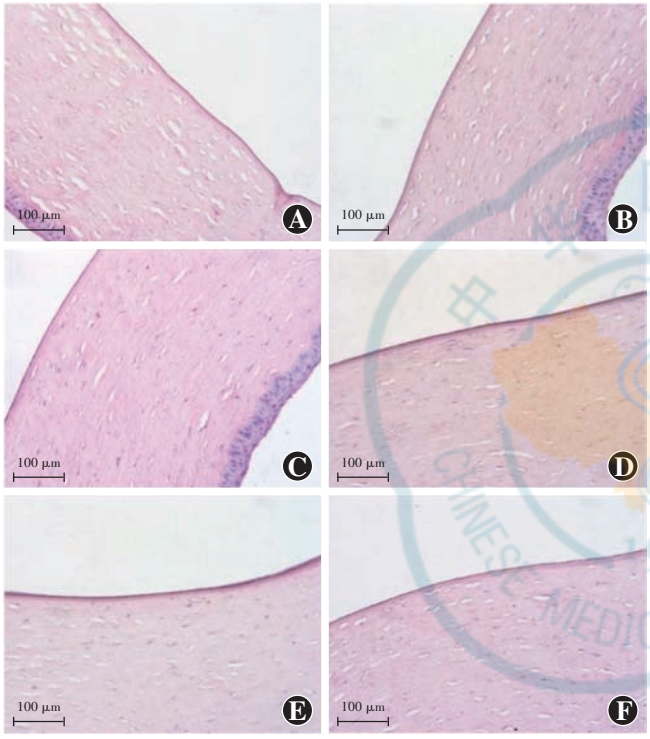

图 4　各组角膜形态学观察（HE ×20，标尺 = 100 μm）　各组注药后不同时间点角膜形态学均未见明显异常　A：玻璃体腔注药组注射后 1 d　B：前房注药组注射后 1 d　C：玻璃体腔注药组注射后 7 d　D：前房注药组注射后 7 d　E：玻璃体腔注射 BSS 后 1 d　F：前房注射 BSS 后 1 d

Figure 4　Corneal morphology of each group（HE ×20, scale bar = 100 μm）　No obvious abnormalities were observed in corneal morphology at different time points after drug injection in each group　A: Intravitreal injection group 1 day post-injection　B: Intracameral injection group 1 day post-injection　C: Intravitreal injection group 7 days post-injection　D: Intracameral injection group 7 days post-injection　E: Intravitreal BSS injection at 1 day post-injection　F: Intracameral BSS injection at 1 day post-injection

玻璃体腔注药组注射后 1 d 角膜内皮细胞肿胀，内质网扩张，部分线粒体肿胀；注射后 7 d 线粒体肿胀仍存在；注射后 14 d，角膜内皮细胞结构恢复正常（图 7）。玻璃体腔注药组注射后 1 d 视网膜组织出现明显水肿，各层细胞间隙扩大。光感受器细胞形态肿胀，排列松散。视网膜神经纤维层可见泡状结构（图 8）。注射后 7 d 视网膜水肿逐渐消退，细胞结构接近正常（图 9）。注射后 28 d 视网膜神经纤维层间仍有组织间液潴留现象（图 10）。

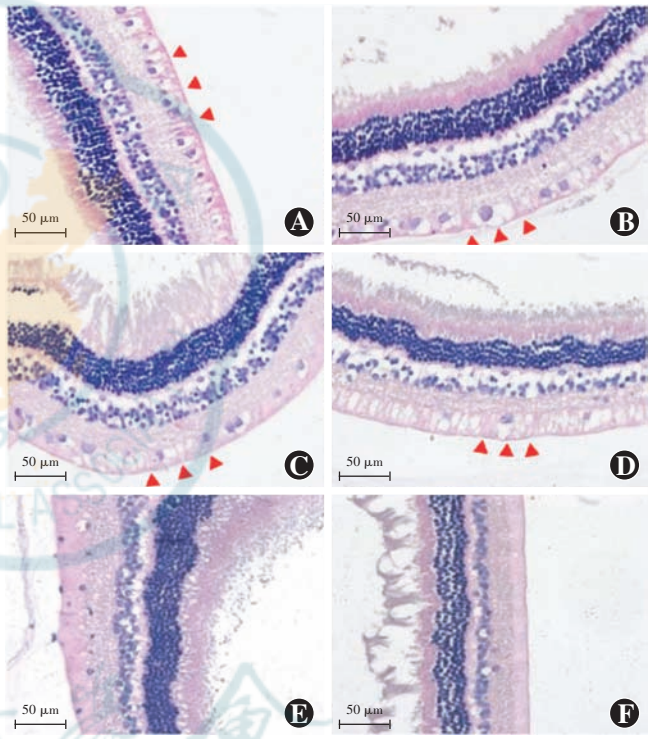

图 5　各组视网膜形态学观察（HE ×40，标尺 = 50 μm）　玻璃体腔注药组和前房注药组注射后 1 和 7 d 均可见视网膜神经纤维层空泡化，伴大量炎性细胞浸润。玻璃体腔注射 BSS 组注射后 1 d 神经纤维层内出现炎性细胞浸润　红色三角示神经纤维层空泡及其内炎性细胞浸润　A：玻璃体腔注药组注射后 1 d　B：前房注药组注射后 1 d　C：玻璃体腔注药组注射后 7 d　D：前房注药组注射后 7 d　E：玻璃体腔注射 BSS 后 1 d　F：前房注射 BSS 后 1 d

Figure 5　Retinal morphological observation of eaeh group（HE ×40, scale bar = 50 μm）　Retinal nerve fiber layer vacuolization accompanied by extensive inflammatory cell infiltration was observed in both the intravitreal injection group and the intracameral injection group on days 1 and 7 post-injection. Inflammatory cell infiltration within the nerve fiber layer was observed 1 day post-injection in the intravitreal BSS injection group. Red triangles indicated nerve fiber layer vacuoles and inflammatory cell infiltration inside　A: Intravitreal injection group 1 day post-injection　B: Intracameral injection group 1 day post-injection　C: Intravitreal injection group 7 days post-injection　D: Intracameral injection group 7 days post-injection　E: Intravitreal BSS injection 1 day post-injection　F: Intracameral BSS injection 1 day post-injection

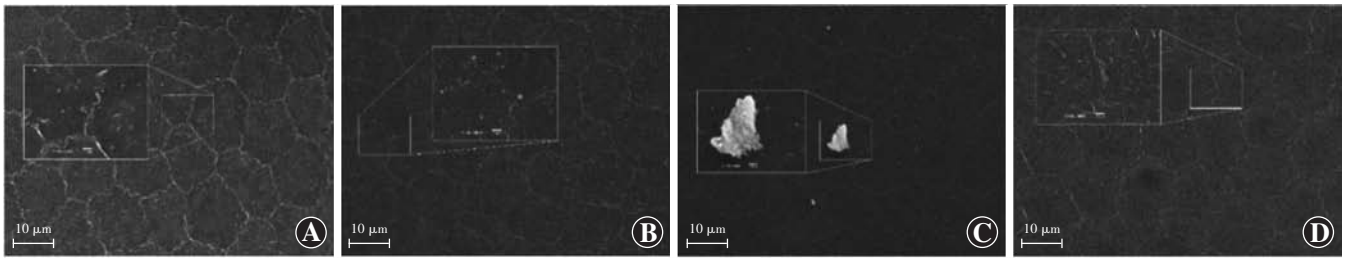

图 6　各组典型角膜内皮细胞扫描电子显微镜图（×1 500，标尺 = 10 μm）　玻璃体腔注药组注射后 1 d 角膜内皮未见明显异常。前房注药组注射后 1 d 不同区域可见大量炎性细胞沉积并黏附在角膜内皮上，注射后 7 d 消失　A：玻璃体腔注药组注射后 1 d　B：前房注药组注射后 1 d　C：不同区域前房注药组注射后 1 d　D：前房注药组注射后 7 d

Figure 6　Scanning electron microscopy images of typical corneal endothelial cells （× 1 500, scale bar = 10 μm）　On day 1 post-injection, no significant abnormalities were observed in the corneal endothelium of the intravitreal injection group. In the intracameral injection group, abundant inflammatory cells were seen deposited to the corneal endothelium in different regions on day 1 post-injection, which disappeared by 7 days post-injection　A: Intravitreal injection group 1 day post-injection　B: Intracameral injection group 1 day post-injection　C: Different regions of intracameral injection group 1 day post-injection　D: Intracameral injection group 7 days post-injection

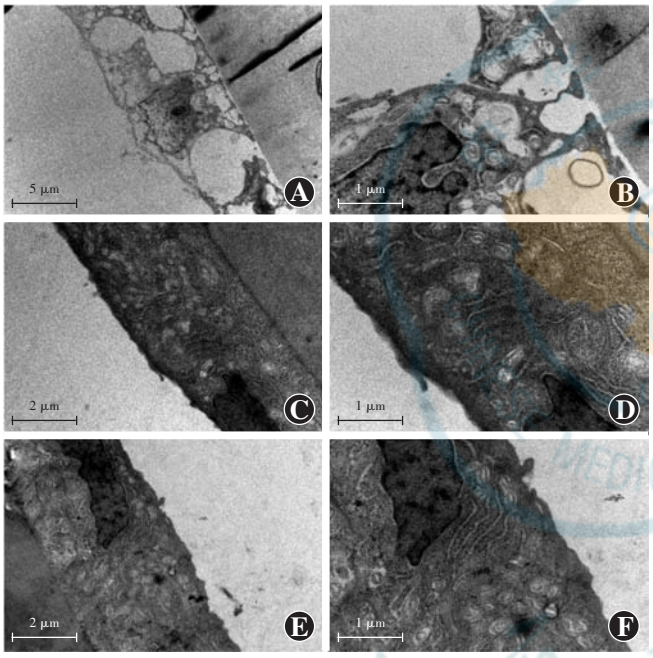

图 7　玻璃体腔注药组注射后不同时间点角膜内皮形态学观察（乙酸双氧铀+柠檬酸铅）　玻璃体腔注药组注射后 1 d 角膜内皮细胞肿胀，内质网扩张，部分线粒体肿胀；注射后 7 d 线粒体肿胀仍存在；注射后 14 d，角膜内皮细胞恢复正常的细胞结构　A：注射后 1 d（标尺 = 5 μm）　B：注射后 1 d（标尺 = 1 μm）　C：注射后 7 d（标尺 = 2 μm）　D：注射后 7 d（标尺 = 1 μm）　E：注射后 14 d（标尺 = 2 μm）　F：注射后 14 d（标尺 = 1 μm）

Figure 7　Morphological observation of corneal endothelial at different time points post-intravitreal injection （uranyl acetate + lead citrate）　On day 1 post-injection, corneal endothelial cells showed swelling, with endoplasmic reticulum dilation and some mitochondrial swelling. Mitochondrial swelling persisted on day 7 post-injection. On day 14 post-injection, corneal endothelial cells had recovered normal cellular structure　A: Day 1 post-injection （scale bar = 5 μm）　B: Day 1 post-injection （scale bar = 1 μm）　C: Day 7 post-injection （scale bar = 2 μm）　D: Day 7 post-injection （scale bar = 1 μm）　E: Day 14 post-injection （scale bar = 2 μm）　F: Day 14 post-injection （scale bar = 1 μm）

前房注药组注射后 1 d 角膜内皮细胞出现明显异常，可见线粒体和内质网肿胀；注射后 7 d 部分线粒体仍然肿胀；注射后 14 d，角膜内皮细胞恢复正常（图 11）。前房注药组注射后 1 d 视网膜组织部分外节膜盘结构混乱，神经纤维层产生组织间液（图 12）；注射后 7 d 视网膜光感受器结构已恢复正常；注射后 28 d 视网膜神经纤维层仍有泡状结构存在（图 13）。

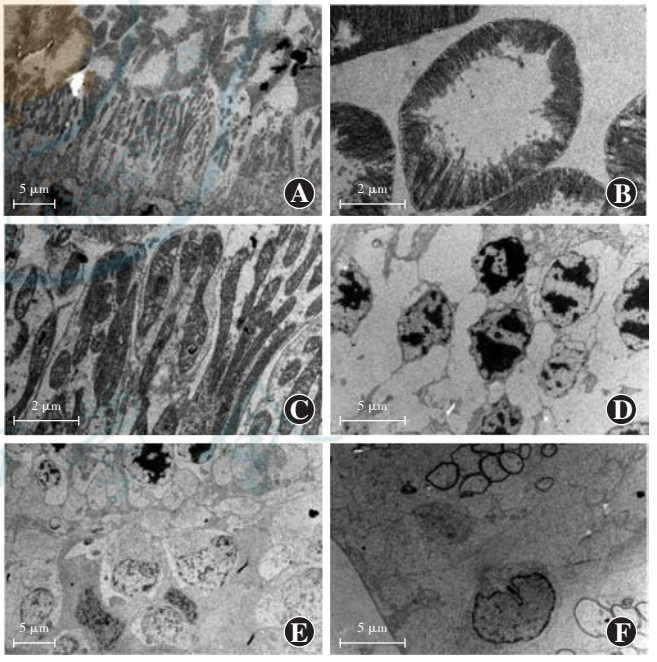

图 8　玻璃体腔注药组注射后 1 d 视网膜不同细胞形态学观察（乙酸双氧铀+柠檬酸铅）　A：光感受器外节和内节（标尺 = 5 μm）　B：光感受器外节和内节（标尺 = 2 μm）　C：光感受器外节和内节（标尺 = 2 μm）　D：外核层（标尺 = 5 μm）　E：内核层（标尺 = 5 μm）　F：视网膜神经纤维层（标尺 = 5 μm）

Figure 8　Morphological observation of different retinal cells on day 1 post-intravitreal injection （uranyl acetate + lead citrate）　A: Photoreceptor outer and inner segments （scale bar = 5 μm）　B: Photoreceptor outer and inner segments （scale bar = 2 μm）　C: Photoreceptor outer and inner segments （scale bar = 2 μm）　D: Outer nuclear layer （scale bar = 5 μm）　E: Inner nuclear layer （scale bar = 5 μm）　F: Retinal nerve fiber layer （scale bar = 5 μm）

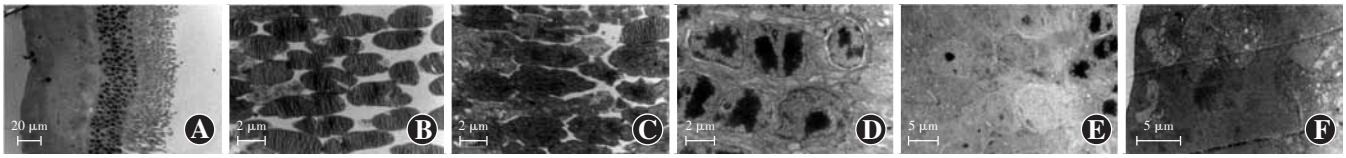

图 9　玻璃体腔注药组注射后 7 d 视网膜不同细胞形态学观察（乙酸双氧铀+柠檬酸铅）　视网膜水肿缓解，结构接近正常　A：视网膜（标尺 = 20 μm）　B：光感受器外节（标尺 = 2 μm）　C：光感受器外节和内节（标尺 = 2 μm）　D：外核层（标尺 = 2 μm）　E：内核层（标尺 = 5 μm）　F：视网膜神经纤维层（标尺 = 5 μm）

Figure 9　Morphological observation of different retinal cells on day 7 post-intravitreal injection (uranyl acetate+lead citrate)　Retinal edema was relieved, retinal structures were normal　A: Retina (scale bar = 20 μm)　B: Photoreceptor outer segment (scale bar = 2 μm)　C: Photoreceptor outer and inner segments (scale bar = 2 μm)　D: Outer nuclear layer (scale bar = 2 μm)　E: Inner nuclear layer (scale bar = 5 μm)　F: Retinal nerve fiber layer (scale bar = 5 μm)

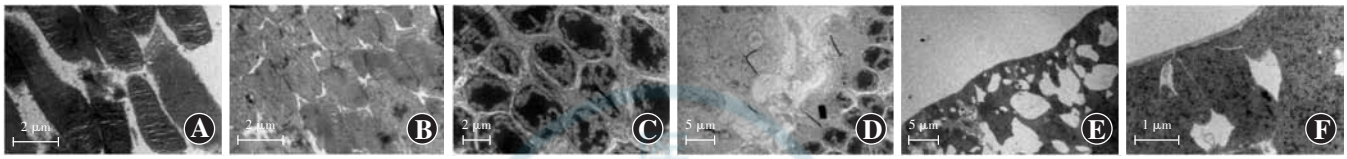

图 10　玻璃体腔注药后 28 d 视网膜不同细胞形态学观察（乙酸双氧铀+柠檬酸铅）　除视网膜神经纤维层中液体潴留（红色三角）外，其余结构大致正常　A：光感受器外节（标尺 = 2 μm）　B：光感受器内节（标尺 = 2 μm）　C：外核层（标尺 = 2 μm）　D：内核层（标尺 = 5 μm）　E：视网膜神经纤维层（标尺 = 5 μm）　F：视网膜神经纤维层（标尺 = 1 μm）

Figure 10　Morphological observation of different retinal cells on day 28 post-intravitreal injection (uranyl acetate+lead citrate)　Retinal structures were normal, with the exception of residual fluid retention in the retinal nerve fiber layer (red triangles)　A: Photoreceptor outer segment (scale bar = 2 μm)　B: Photoreceptor inner segment (scale bar = 2 μm)　C: Outer nuclear layer (scale bar = 2 μm)　D: Inner nuclear layer (scale bar = 5 μm)　E: Retinal nerve fiber layer (scale bar = 5 μm)　F: Retinal nerve fiber layer (scale bar = 1 μm)

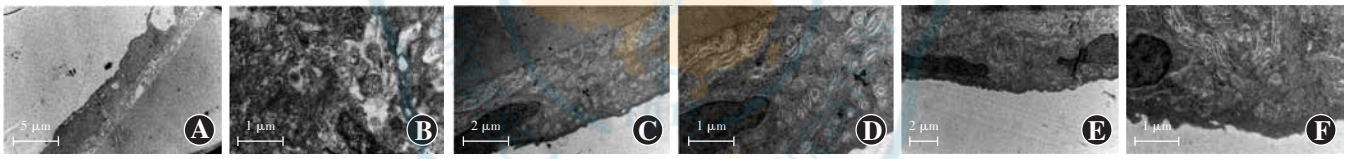

图 11　前房注药组注射后不同时间点角膜内皮形态学观察（乙酸双氧铀+柠檬酸铅）　注射后 1 d 角膜内皮细胞出现线粒体和内质网肿胀，注射后 7 d 部分线粒体仍然肿胀，注射后 14 d 结构恢复正常　A：注射后 1 d（标尺 = 5 μm）　B：注射后 1 d（标尺 = 1 μm）　C：注射后 7 d（标尺 = 2 μm）　D：注射后 7 d（标尺 = 1 μm）　E：注射后 14 d（标尺 = 2 μm）　F：注射后 14 d（标尺 = 1 μm）

Figure 11　Morphological observation of corneal endothelium at different time points post-intracameral injection (uranyl acetate+lead citrate)　On day 1 post-injection, mitochondrial and endoplasmic reticulum swelling was observed in corneal endothelial cells. Mitochondria remained swollen on day 7 post-injection and returned to normal on day 14 post-injection　A: Day 1 post-injection (scale bar = 5 μm)　B: Day 1 post-injection (scale bar = 1 μm)　C: Day 7 post-injection (scale bar = 2 μm)　D: Day 7 post-injection (scale bar = 1 μm)　E: Day 14 post-injection (scale bar = 2 μm)　F: Day 14 post-injection (scale bar = 1 μm)

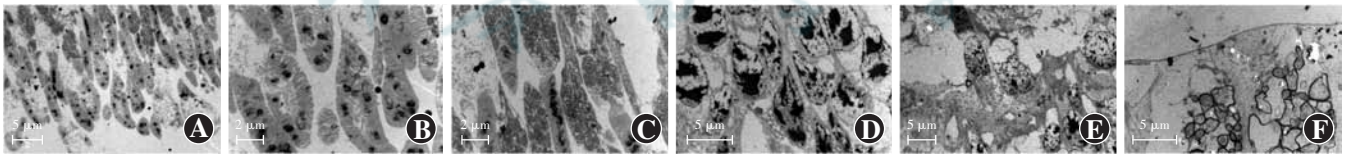

图 12　前房注药组注射后 1 d 视网膜不同细胞形态学观察（乙酸双氧铀+柠檬酸铅）　注射后 1 d 视网膜光感受器外节、内节、外核层、内核层均出现水肿，神经纤维层可见大量泡状结构　A：光感受器外节（标尺 = 5 μm）　B：光感受器外节（标尺 = 2 μm）　C：光感受器内节（标尺 = 2 μm）　D：外核层（标尺 = 5 μm）　E：内核层（标尺 = 5 μm）　F：视网膜神经纤维层（标尺 = 5 μm）

Figure 12　Morphological observation of different retinal cells in the intracameral injection group on day 1 post-injection (uranyl acetate+lead citrate)　Edema was observed in the retinal photoreceptor outer segment, photoreceptor inner segment, outer nuclear layer, and inner nuclear layer. Abundant vacuolar structures were visible in the nerve fiber layer　A: Photoreceptor outer segment (scale bar = 5 μm)　B: Photoreceptor outer segment (scale bar = 2 μm)　C: Photoreceptor inner segment (scale bar = 2 μm)　D: Outer nuclear layer (scale bar = 5 μm)　E: Inner nuclear layer (scale bar = 5 μm)　F: Retinal nerve fiber layer (scale bar = 5 μm)

3　讨论

眼内病毒感染易复发，尤其是对于某些难治性病例，现有抗病毒药物往往难以发挥作用，治疗不及时可能导致永久性视力损害，寻找安全有效的抗病毒疗法一直是眼科领域面临的重大挑战。

近年来，玻璃体腔注射膦甲酸钠作为一种新型抗病毒治疗手段，在巨细胞病毒性视网膜炎的治疗中逐

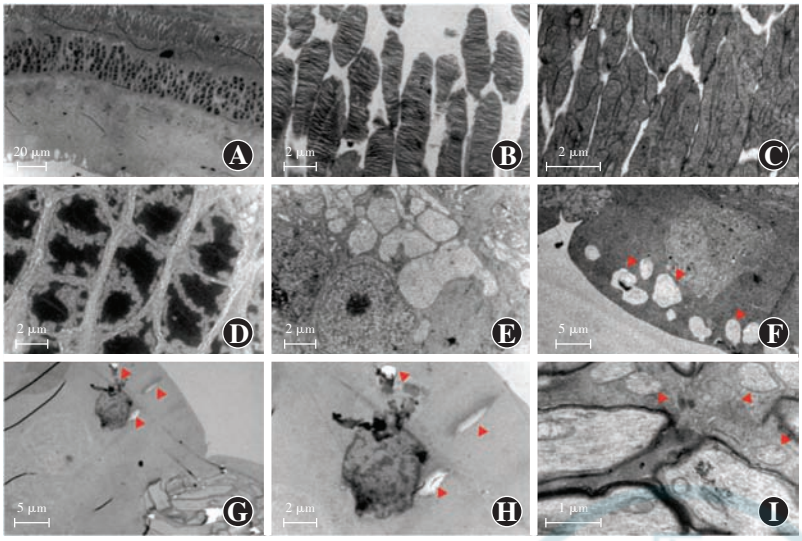

图 13　前房注药组注射后 7 d 及 28 d 视网膜不同细胞形态学观察（乙酸双氧铀+柠檬酸铅）　前房注药后 7 d 视网膜神经纤维层仍有液体潴留，视网膜其余各层结构大致正常；前房注药后 28 d 视网膜神经纤维层中泡状结构（红色三角）较前减少　A：7 d 后视网膜（标尺=20 μm）　B：7 d 后光感受器外节（标尺=2 μm）　C：7 d 后光感受器内节（标尺=2 μm）　D：7 d 后外核层（标尺=2 μm）　E：7 d 后内核层（标尺=2 μm）　F：7 d 后视网膜神经纤维层（标尺=5 μm）　G：28 d 后视网膜神经纤维层中泡状结构减少（标尺=5 μm）　H：28 d 后视网膜神经纤维层中泡状结构减少（标尺=2 μm）　I：28 d 后视网膜神经纤维层中泡状结构（标尺=1 μm）

Figure 13　Morphological observation of different retinal cells in the intracameral injection group on days 7 and 28 post-injection (uranyl acetate+lead citrate)　On day 7 post-injection, fluid retention was still present in the retinal nerve fiber layer, and the remaining retinal layers exhibited largely normal structures. On day 28 post-injection, the vacuolar structures (red triangles) within the retinal nerve fiber layer were reduced compared to earlier　A: Retina on day 7 (scale bar=20 μm)　B: Photoreceptor outer segment on day 7 (scale bar=2 μm)　C: Photoreceptor inner segment on day 7 (scale bar=2 μm)　D: Outer nuclear layer on day 7 (scale bar=2 μm)　E: Inner nuclear layer on day 7 (scale bar=2 μm)　F: Retinal nerve fiber layer on day 7 (scale bar=5 μm)　G: Reduced vacuolar structures in the retinal nerve fiber layer on day 28 (scale bar=5 μm)　H: Reduced vacuolar structures in the retinal nerve fiber layer on day 28 (scale bar=2 μm)　I: Vacuolar structures in the retinal nerve fiber layer on day 28 (scale bar=1 μm)

渐展露其独特优势。该方法通过将药物直接注射到玻璃体腔中，从而有效绕过眼部屏障，迅速提高眼内药物浓度，减少全身不良反应的同时提高疗效，主要适用于无法耐受全身治疗或对更昔洛韦等传统治疗药物不敏感的患者[6,21]。尽管玻璃体腔注射膦甲酸钠具有一系列优点，但其侵入性和其他相关并发症不容忽视。目前，关于玻璃体腔注射膦甲酸钠治疗病毒性角膜内皮炎的基础研究尚不完善，其对于眼内组织，特别是对于角膜内皮的安全性仍不清楚。

目前尚无研究探讨玻璃体腔注射膦甲酸钠在病毒性角膜内皮炎治疗中的应用，本研究以巨细胞病毒性视网膜炎的治疗方案作为参考。临床上治疗巨细胞病毒性视网膜炎时常采用每周 2 次玻璃体腔注射膦甲酸钠，每次剂量为 0.1 ml 溶液含膦甲酸钠 2.4 mg[21]。考虑到人眼玻璃体容积是兔眼的 2.5～3 倍，视网膜表面积是兔眼的 2 倍，且即使玻璃体腔注射技术娴熟，在取针时也难以避免有极少量注射液从眼中溢出，因此本研究选择 1.2 mg 的剂量进行兔眼内注射。

Berthe 等[22]研究提示，兔眼玻璃体腔内注射 0.1 ml 膦甲酸钠 2.4 mg 或混合注射膦甲酸钠 1.2 mg 和更昔洛韦 0.5 mg 后，均未观察到振荡电位或视网膜电图 a 波、b 波的异常改变，组织学观察视网膜各层结构正常，未见细胞病理改变。Turrini 等[23]研究发现，随着注射次数的增加，相关损伤逐渐显现，接受 2 次玻璃体腔注射 0.1 ml 膦甲酸钠 2.4 mg 的兔眼与注药前或玻璃体腔注射 BSS 的对照组相比，各项视网膜电图相关参数比较差异均无统计学意义；接受 4 次玻璃体腔注药的兔眼与对照组相比，暗适应下视网膜电图相关参数显著下降；接受 6 次玻璃体腔注药，与注药前或与对照组相比，中等光照及暗适应条件下视网膜电图检查均提示各参数显著下降。接受至少 4 次玻璃体腔注药的兔眼出现光感受器层和内核层轻微空泡变性；而接受 6 次注射的眼中，可见光感受器层的局灶性破坏[23]。此外，动物研究表明膦甲酸钠实验性治疗单纯疱疹病毒 1 型视网膜炎效果良好，且 0.1 ml 溶液中高达 1 mg 的药物剂量对兔视网膜无毒性作用[24]。

在本研究中，玻璃体腔注射膦甲酸钠后眼前节裂隙灯显微镜和光学显微镜下观察可见角膜内皮与注药前相比均无显著差异。透射电子显微镜下观察结果显示，玻璃体腔注射后第 1 天出现内皮细胞肿胀和视网膜水肿，但随时间的推移，组织水肿逐渐消退，角膜内皮细胞结构恢复正常，这些结果表明玻璃体腔注射 1.2 mg 膦甲酸钠对角膜有短暂毒性作用。考虑到人角膜内皮细胞无法增殖的特性，对于玻璃体腔反复注射膦甲酸钠应持谨慎态度。

本研究中 2 种注药方式在光学显微镜及透射电子显微镜下均观察到视网膜各层结构出现水肿，随时间延长光感受器细胞结构恢复正常，但神经纤维层仍然存在部分组织间液残留，至观察终止尚未完全消退。本研究中视网膜组织水肿等组织学损伤出现于单次注药后 1 d，与既往研究相比，组织损伤性质具有相似性，但出现更早，考虑可能与不同的药物生产厂家、批次有关，后续仍需更长时间的实验观察。本研究聚焦于观察视网膜及角膜的结构性改变，未来需进一步研

究不同浓度梯度的药物毒性作用、视网膜电生理改变等，并可扩大研究范围至眼内其他组织（如晶状体）以及对全身脏器等的潜在影响。

本研究首次评估了前房注射膦甲酸钠的眼毒性，裂隙灯显微镜检查和细胞结构检查均显示角膜水肿，透射电子显微镜检查结果也显示了注射后短时间光感受器的变化。与本课题组之前的研究相比，尽管前房注射膦甲酸钠比注射更昔洛韦导致的角膜水肿和内皮损伤程度明显较轻[25]，然而考虑到房水循环的速度，本研究不建议将膦甲酸钠直接注入前房。

综上，本研究系统性评价了膦甲酸钠眼内注射对角膜的毒性，结果显示膦甲酸钠 2 种注药方式对视网膜存在短暂毒性作用，随时间延长作用逐渐减弱；前房内注射膦甲酸钠对角膜有一定的短暂毒性作用，对视网膜的毒性随时间延长逐渐减轻，其毒性作用较玻璃体腔内注药更显著，考虑到人角膜内皮不可再生，因此临床应用需十分谨慎。

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