Effective early antiviral treatments reduce both acute zoster pain and the risk of postherpetic neuralgia. The authors hypothesized that the direct neuraxial administration of acyclovir could provide superior drug application to the spinal neural structures with a higher viral burden and have various advantages over the other routes of drug administration in terms of required doses, side effects, and efficacy.

**Objective** To know whether intrathecal acyclovir injection is neurologically and histopathologically safe or not.

**Study Design:** Randomized, experimental trial in rats.

**Setting:** Associated experiment center.

**Methods:** A total of 40 rats weighing between 250-300g were used. The rats were randomly divided into 2 groups of 20 each using a random number table: normal saline group (Group N) and acyclovir group (Group A). Rats in Group N were administered 20 µl of normal saline and Group A were administered the same volume of 700 µg/mL acyclovir into the intrathecal space via an intrathecal catheter. Saline or acyclovir was administered daily for 5 consecutive days. The changes in behavior and sensory-motor function were checked and histopathological findings of the spinal cords were observed by light and electron microscopy.

**Results:** No rats in Group N or Group A showed any behavioral change or sensory-motor dysfunction during the 5-day observation period. Furthermore, no histopathological abnormalities of the spinal cord were observed in the 6th day after the last intrathecal administration of the drug.

**Limitations:** There is a need to perform studies to evaluate long-term safety by observing cumulative neurotoxic effects with continual injection during a long-term period.

**Conclusions:** There was no evidence of neurological and histopathological abnormalities following intrathecal acyclovir injection.

**Key words:** acyclovir, safety, spinal, intrathecal, acute zoster pain, post herpetic neuralgia

Although herpes zoster is not a fatal disease, it can cause severe zoster-related pain including both acute zoster pain and chronic pain of post herpetic neuralgia (PHN). Severe zoster-related pain can cause physical disability and emotional distress, impair quality of life, and create an economic burden on the individual and society. It is well known that antiviral treatments are available to ameliorate acute zoster pain and to prevent PHN (1-3). Based on the available literature, drug administration to
spinal neural structures is superior than others (4-14). Consequently, in this study, we sought to evaluate the neurological safety of intrathecal acyclovir by observing the changes in behavior, sensory-motor function, and histopathological findings of the rat spinal cords by light and electron microscopy before conducting a clinical trial in humans.

**Methods**

Male Sprague–Dawley rats weighing 250–300 g were purchased from Dual Laboratories, Inc. (Seoul, Republic of Korea). All rats were used under standardized environmental conditions (12-hour light/dark cycle, 21% and 55% humidity) and acclimatized for at least 5 days before the experiment. Animals were allowed free access to food and water throughout. The experimental protocol used was reviewed and approved by the Animal Care and Use Committee at Korea University. Every measure was taken to minimize animal discomfort. Anesthesia was induced by placing rats individually in a closed box containing 4% enflurane in oxygen (3 L/min). After loss of consciousness, anesthesia was maintained with 2%-3% enflurane via a loose-fitting mask. After applying a sterile dressing, the subarachnoid space was cannulated with a PE-10 catheter (0.28 mm inner diameter, 0.61 mm outer diameter, [Becton Dickson, Sparks, MD]) through the atlanto-occipital membrane using a modification of the method described by Yaksh and Rudy (15,16).

The tip of the catheter was gently advanced about 7 cm in the caudal direction, which placed it at approximately the L2 level. The other end of the catheter was fixed by suturing it to subcutaneous tissue to avoid dislocating the indwelling catheter. The fascia and skin were sutured and antibiotic ointment was applied. The rats were allowed to recover for 5 days before drug administration. Rats that showed symptoms of spinal cord injury were excluded from further experiments. Using a random number table, 40 rats were randomly and equally allocated into 2 groups, the normal saline placebo group (Group N, n = 20) and the acyclovir group (Group A, n = 20) after 5 days of intrathecal catheterization. Rats in Group N received 20 µl of normal saline (Group A, n = 20) after 5 days of intrathecal catheterization. Rats in Group N received 20 µl of normal saline (Group A, n = 20) after 5 days of intrathecal catheterization.

Approximately one cm lengths of spinal cord, caudal and rostral to the catheter tip, were obtained. For light microscopy, spinal cords were fixed with 10% neutral formalin solution. Using standard tissue slide preparation methods, 3 blocks were made per sample. The slides were prepared from 3µm sections stained with hematoxylin and eosin. To evaluate myelin and axonal destruction, Luxol fast blue (a specific myelin stain) and Bodian stain were added. For elec-
electron microscopic examinations, tissue samples were sliced into one mm3 blocks, and fixed in 2.5% glutaraldehyde solution for 24 hours at 1–4°C. Tissues were then washed with 0.1 M phosphate buffer solution, submerged in 1% OsO4 solution, washed with phosphate buffer solution, dehydrated using an alcohol series, immersed in propylene oxide, embedded in epoxy resin using the Luft method, and finally thermally polymerized at 80°C for 24 hours. Prepared tissues were sectioned at 1 µm, stained with toluidine-O, and viewed under a light microscope. In addition, 50–60 nm sections were prepared using an ultramicrotome, attached to a grid, electronically stained with alanineacetate and lead citrate, and then observed under a transmission electron microscope (H-7100, Hitachi, Tokyo, Japan).

Statistical Analysis

Statistical analysis was performed using SigmaStat for Windows version 3.0.1a (Systat Software Inc, Chicago, IL) and SPSS 15.0 for Windows (SPSS Inc. Chicago, IL). Statistical significance was accepted for P values of < 0.05. The run test was performed to confirm the randomness of group allocations, and repeated measures analysis of variance (ANOVA) was used to analyze longitudinal results. Bonferroni’s t-test was used for multiple comparisons when P values determined by repeated measures ANOVA indicated significant differences. The Student’s t-test was used to compare the 2 study groups at given time points. All results are expressed as means and standard deviations.

**Results**

Forty-four rats were prepared for intrathecal catheterization, but 4 rats were excluded due to motor deficits after cannulation. To ensure the randomness of animal group allocations, allocation numbers were tested using a run test. The P value of 0.920 obtained indicated that the randomization procedure was adequate. The thermal latency withdrawal test results of both study groups showed no definite changes during the 5 day observation period (P values 0.370 and 0.780 for the N and A groups, respectively, Table 1). Furthermore, test results at given time points showed no differences in the two groups; P values at pre-administration (PRE), and on days 1 to 5 were 0.722, 0.093, 0.073, 0.764, 0.487, and 0.401, respectively. Similarly, the tactile allodynia test results of both study groups showed no definite changes during the 6 day observation period (P values 0.858 and 0.796 for the N and A groups, respectively, Table 2). No significant intergroup differences in 50% threshold values were observed between given time points, and corresponding P values at PRE, and at days 1 to 5 were 0.693, 0.484, 0.364, 0.733, 0.827, and 0.393, respectively. Body weights during the observation period showed a similar trend in the 2 groups (Table 3). On the day after intrathecal catheterization, body weights decreased significantly versus baseline and then gradually increased (P < 0.001 in each group). Intergroup differences in body weight were not observed at given time points (P values at PRE and at 1 to 5 days were 0.944, 0.446, 0.662, 0.693, 0.897, 0.989,

### Table 1. Thermal withdrawal latency test

<table>
<thead>
<tr>
<th></th>
<th>Group N</th>
<th></th>
<th>Group A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Initial</td>
<td>6.67</td>
<td>1.083</td>
<td>6.789</td>
<td>1.017</td>
</tr>
<tr>
<td>1 day</td>
<td>6.609</td>
<td>0.934</td>
<td>6.736</td>
<td>0.844</td>
</tr>
<tr>
<td>2 day</td>
<td>6.675</td>
<td>0.723</td>
<td>6.691</td>
<td>0.817</td>
</tr>
<tr>
<td>3 day</td>
<td>6.595</td>
<td>0.589</td>
<td>6.713</td>
<td>0.812</td>
</tr>
<tr>
<td>4 day</td>
<td>6.941</td>
<td>0.901</td>
<td>6.786</td>
<td>0.78</td>
</tr>
<tr>
<td>5 day</td>
<td>6.825</td>
<td>0.98</td>
<td>6.911</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation of data. There were no significant differences between values of group N and group A, also no differences in values in each group. Group N = normal saline group; group A = acyclovir group.

### Table 2. Tactile allodynia test

<table>
<thead>
<tr>
<th></th>
<th>Group N</th>
<th></th>
<th>Group A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>Initial</td>
<td>14.60</td>
<td>0.88</td>
<td>14.49</td>
<td>0.89</td>
</tr>
<tr>
<td>1 day</td>
<td>14.78</td>
<td>0.46</td>
<td>14.67</td>
<td>0.52</td>
</tr>
<tr>
<td>2 day</td>
<td>14.71</td>
<td>0.85</td>
<td>14.42</td>
<td>1.13</td>
</tr>
<tr>
<td>3 day</td>
<td>14.43</td>
<td>1.25</td>
<td>14.30</td>
<td>1.31</td>
</tr>
<tr>
<td>4 day</td>
<td>14.48</td>
<td>1.12</td>
<td>14.55</td>
<td>0.89</td>
</tr>
<tr>
<td>5 day</td>
<td>14.54</td>
<td>0.88</td>
<td>14.24</td>
<td>1.30</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation of data. There were no significant differences between values of group N and group A, also no differences in values in each group. Group N = normal saline group; group A = acyclovir group.
and 0.736, respectively). Tissue analysis under light microscopy showed no local neuritis, meningeal inflammation, local myelopathy, or myelin loss. And the electron microscopic findings of both groups revealed that no microstructural changes were found in neurons, glial cells or endothelial cells (Figs. 1, 2).

Table 3. Body weight changes

<table>
<thead>
<tr>
<th></th>
<th>Group N</th>
<th></th>
<th>Group A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard Deviation</td>
<td>Mean</td>
</tr>
<tr>
<td>Initial</td>
<td>276.70</td>
<td>15.35</td>
<td>276.35</td>
</tr>
<tr>
<td>Post Surgery</td>
<td>262.05*</td>
<td>15.80</td>
<td>258.30*</td>
</tr>
<tr>
<td>1 day</td>
<td>260.80*</td>
<td>14.44</td>
<td>258.85*</td>
</tr>
<tr>
<td>2 day</td>
<td>262.80*</td>
<td>13.09</td>
<td>264.25*</td>
</tr>
<tr>
<td>3 day</td>
<td>265.40*</td>
<td>13.24</td>
<td>265.90*</td>
</tr>
<tr>
<td>4 day</td>
<td>269.00*†‡§</td>
<td>12.04</td>
<td>268.95*</td>
</tr>
<tr>
<td>5 day</td>
<td>270.20*†‡§</td>
<td>10.88</td>
<td>271.40†‡§</td>
</tr>
</tbody>
</table>

Values are expressed as mean and standard deviation of data; measurement unit is gram.
Statistical differences (P < 0.05) are marked as follows: * vs initial values; † vs post surgery; ‡ vs 1 day; § vs 2 day. Group N = normal saline group; Group A = acyclovir group.

**Hematoxylin and eosin staining.**
Sections of animals in the acyclovir and saline study groups showed a normal anatomical architecture with well-preserved ventral and dorsal horns. No pathologic neuronal changes were observed. The mild vacuolar changes present in white matter are due to artifacts. Original magnification 40X.

**Luxol-Fast Blue staining**
At one day after the last intrathecal injection of saline (left) or acyclovir (right). The myelin fibers in both study groups were normal. Luxol-Fast Blue stain. Original magnification 100X.

**Bodian staining**
At one day after the last intrathecal injection of saline (left) or acyclovir (right), the fibrillary structures of axons were found to be well preserved. Bodian stain, which stains axons black; original magnification 400X.
Neurological Safety of Intrathecal Acyclovir in Rats

**Discussion**

Zoster-related pain including PHN remains a challenge for effective management of zoster infection. Herpes zoster arises when dormant virus particles, persisting within an affected trigeminal or dorsal root ganglion from an earlier primary infection with varicella, become reactivated when cellular immunity to varicella decreases with advancing age or immunosuppressive diseases and treatments such as human immunodeficiency virus infection, malignancy, or chemotherapy (3, 19). Viral particles replicate and spread transaxially to the dorsal root, the dorsal horn of the spinal cord, and through peripheral sensory nerve fibers to the skin (20, 21). The reactivated virus destroys affected central and peripheral nerves and leads to inflammation, immune response, hemorrhagic necrosis, and neuronal loss to varying extents within affected spinal ganglia (22-24). Typical intranuclear viral inclusions and viral particles are also detectable by light and electron microscopy. Considering all these neuropathologic phenomena, early treatment with antiviral agents can be the most important therapeutic keystone in the treatment of herpes zoster. Antiviral agents are actually effective to control zoster-related pain and to prevent its complications. Antiviral agents attenuate the severity of zoster, the duration of viral shedding, acute pain, rash healing, and the incidence of PHN (1, 25-29). Acyclovir is a synthetic acyclic purine nucleoside analogue derived from guanine and used in the treatment of herpes simplex, herpes zoster, and varicella zoster virus infections. Acyclovir is converted to its active form by herpes simplex and varicella-zoster specified thymidine kinase. The activated acyclovir interferes with viral DNA synthesis and inhibits viral replication (30, 31). The concentration of acyclovir required to inhibit by 50% the growth of most laboratory strains and clinical isolates of varicella zoster virus in cell culture (IC50) ranges from 0.12 to 10.8 μg/mL. This should be given a number and added to the reference list. It was previously reported that acyclovir concentrations in cerebrospinal fluid (CSF) following oral administration is only 13 to 52% of the concentrations in serum at steady state (32). Generally, neuraxial drug administration bypasses the blood-brain barrier and provides better access to spinal neural structures only with very small doses (5, 6, 33). We hypothesized that direct neuraxial administration of antiviral agents could provide superior drug application to the spinal neural structures with the highest viral burden and also minimize unwanted dose-dependent side effects in a certain clinical status such as renal failure (34-38). Moreover, we postulated that neuraxial administration of these agents could also minimize unwanted dose-dependent side effects in a certain clinical status such as renal failure (34-38). Neuraxial injection of antiviral agents has not been previously reported. We undertook the present study to confirm the safety of neuraxial acyclovir administration with overdoses by observing neurological safety after an intrathecal acyclovir antiviral agent injection in rats. We chose pure acycloguanosine without preservatives or other organic solvents and a maximum concentration of acycloguanosine of 700 μg/mL. We thought this concentration was

**Fig. 2.** Electron microscopic findings of spinal cords after acyclovir and saline injection in rats. In both cases, axons were well surrounded with myelin and there was no evidence of demyelination or degenerative change. No microstructural changes were found in neurons, glial cells, or endothelial cells. Original magnification 30,000X.
sufficient to prove the safety of intrathecal injections before clinical application. The concentration of acyclovir used in this study was several hundred times higher than that reported for preventing virus replication of varicella zoster. The present study has a limitation that needs to be taken into account. Although the study observed the neurological safety following intrathecal administration of acyclovir daily for 5 consecutive days, there is a need to perform studies to evaluate long-term safety by observing the cumulative neurotoxic effects with continual injections during a long-term period.

Conclusions

There was no evidence of neurological abnormality following daily intrathecal acyclovir injection with overdose for 5 days in rats. The present study will provide impetus for further trials before any clinical trial of neuraxial antiviral injection in humans.

References
