Experimental Trial

The Neurological Safety of Intrathecal Acyclovir in Rats

Heezoo Kim MD, PhD[1], Yoon-La Choi, MD, PhD[2], Dong Kyu Lee MD, PhD[1], Sang Sik Choi MD, PhD[1], Il Ok Lee MD, PhD[1], Myoung Hoon Kong, MD, PhD[1], Nan Sook Kim MD, PhD[1], Sang Ho Lim MD, PhD[1], and Mikyoung Lee, MD, PhD[1]

From: [1]Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, South Korea; [2]Department of Pathology, Samsung Medical Center, Sungkyunkwan University, School of Medicine, Seoul, Korea

Dr. Kim, Dr. DK Lee, and Dr. SS Choi are Assistant professors with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, South Korea. Dr. YL Choi is an Assistant Professor with the Department of Pathology, Samsung Medical Center, Sungkyunkwan University, School of Medicine, Seoul, Korea
Dr. IO Lee, Dr. Kong, Dr. NS Kim, Dr. Lim and Dr. M Lee are professors with the Department of Anesthesiology and Pain Medicine, Korea University Guro Hospital, Seoul, South Korea.

Address correspondence:
Sang Sik Choi, MD, PhD,
Department of Anesthesiology and Pain Medicine
Korea University Guro Hospital
Guro-Dong 80, Guro-gu
Seoul, South Korea, 152-703
E-mail: clonidine@empal.com

Disclaimer: There was no external funding in the preparation of this manuscript.
Conflict of interest: None.

Manuscript received: 07/16/2011
Revised manuscript received: 09/26/2011
Accepted for publication: 10/06/2011

Free full manuscript:
www.painphysicianjournal.com

Background: 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

Pain Physician 2012; 15:E107-E113

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 evalute 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 and rats in Group A received the same volume of 700 µg/mL acyclovir into the intrathecal space daily for 5 consecutive days. Immediately before and 60 minutes after each daily injection, body weights, a motor function test, a thermal test, and von Frey test were performed. Rats were sacrificed by transcardial perfusion with 4% paraformaldehyde in 0.1 molar concentration phosphate buffer under general anesthesia on day 6 to analyze histopathologic spinal cord changes. Histopathological examinations were performed under light and electron microscopes. All evaluations and measurements were performed by an independent researcher unaware of group and study details.

Motor- function test: Motor function was assessed using a previously devised scoring system with some modification (17). Grades were defined as follows: Grade 1 = normal gait with no evidence of motor paresis; Grade 2 = normal gait with slight hind paw deformity, such as, plantar flexion of toes; Grade 3 = slight gait disturbance with motor weakness and/or an inverted hind paw; and Grade 4 = a prominent limping gait with a dropped hind paw. The rats with a motor disturbance of Grade 2 or above were considered to have a motor deficit.

Thermal withdrawal latency test: Heat was delivered to the plantar surfaces of hind limbs using a hot plate. The latency of hind limb withdrawal response to radiant heat was measured differences of latency between before and after injecting the test drug. Measurements were repeated 5 times on both hind paws of each rat. The maximum exposure time was fixed at 15 seconds to prevent thermal injury.

Von Frey examination: Mechanical threshold of tactile allodynia was measured by applying 8 calibrated von Frey hairs (0.41-5.10 g) to the plantar surfaces of both hind feet. Briefly, each rat was placed in a transparent plastic dome (28 cm × 28 cm × 10 cm) with a metal mesh floor, and a von Frey filament was applied for 3-4 seconds with enough pressure to bend it slightly. Brisk withdrawal and paw flinching were considered as positive responses. Filaments with gradually increasing bending strengths were applied until a positive response was obtained. Fifty percent withdrawal thresholds were determined using the up-down method (18).

On day 6, the final study day, rats were sacrificed under deep anesthesia (thiopentone sodium, 35 mg/kg intraperitoneally) by transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer. 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-

tron microscopic examinations, tissue samples were sliced into one mm3 blocks, and fixed in 2.5% glutaldehyde 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 alanyl-acetate 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*

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

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*

	Group N		Group A	
	Mean	Standard Deviation	Mean	Standard Deviation
Initail	14.60	0.88	14.49	0.89
1 day	14.78	0.46	14.67	0.52
2 day	14.71	0.85	14.42	1.13
3 day	14.43	1.25	14.30	1.31
4 day	14.48	1.12	14.55	0.89
5 day	14.54	0.88	14.24	1.30

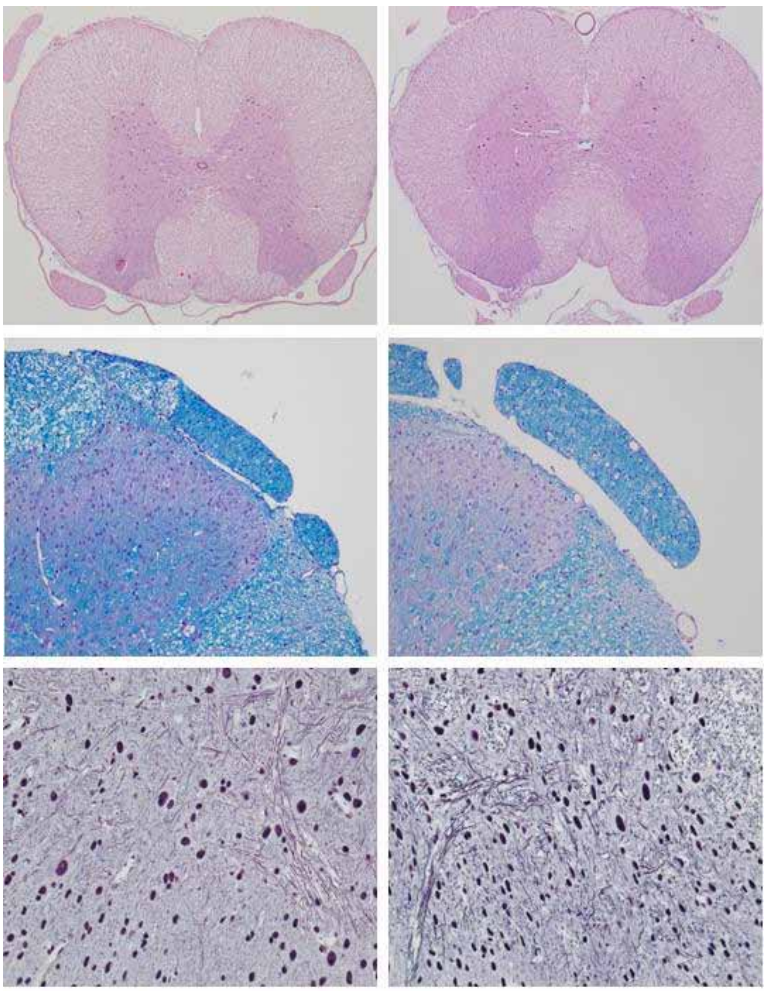
Values are expressed as mean and standard deviation of data.
There were no significant differences between values of group N and group A, also no differences in values in each group. Group N = normal saline group; group A = acyclovir group.

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*

	Group N		Group A	
	Mean	**Standard Deviation**	**Mean**	**Standard Deviation**
Initail	276.70	15.35	276.35	15.88
Post Surgery	262.05*	15.80	258.30*	14.95
1 day	260.80*	14.44	258.85*	13.50
2 day	262.80*	13.09	264.25*	9.75
3 day	265.40*	13.24	265.90*†‡	10.83
4 day	269.00*†‡§	12.04	268.95*†‡	11.78
5 day	270.20*†‡§	10.88	271.40†‡§	11.50

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.

Fig. 1. *Light microscopic findings of spinal cord cross section.*

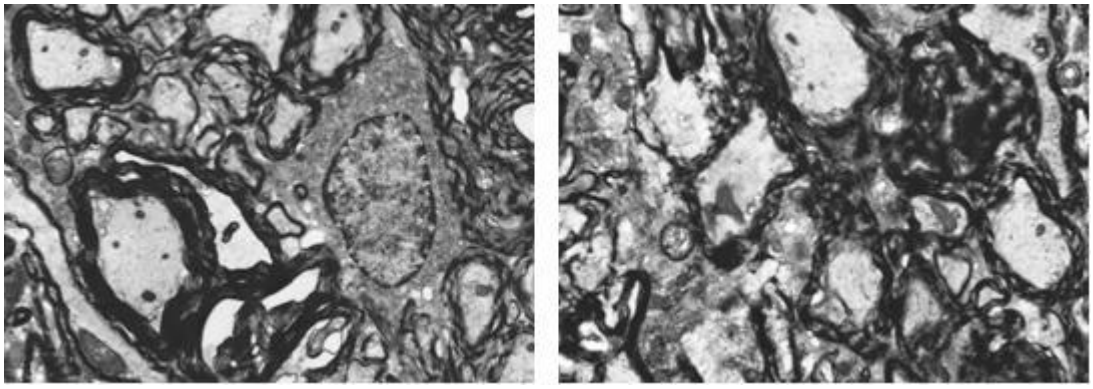


Fig. 2. *Electron microscopic findings of spinal cords after acyclovir and saline injection in rats. In both cases, axons were well surrounded with myelin and there was no evidence of demyelinzation or degenerative change. No microstructural changes were found in neurons, glial cells, or endothelial cells. Original magnification 30,000X.*

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 transaxonally 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

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

1. Lilie HM, Wassilew S. The role of antivirals in the management of neuropathic pain in the older patient with herpes zoster. *Drugs Aging* 2003; 20:561-570.
2. Mustafa MB, Arduino PG, Porter SR. Varicella zoster virus: review of its management. *J Oral Pathol Med* 2009; 38:673-688.
3. Schmader KE, Dworkin RH. Natural history and treatment of herpes zoster. *J Pain* 2008; 9:S3-S9.
4,. Nordberg G, Hedner T, Mellstrand T, Dahlstrom B. Pharmacokinetic aspects of epidural morphine analgesia. *Anesthesiology* 1983; 58:545-551.
5. Osenbach RK, Harvey S. Neuraxial infusion in patients with chronic intractable cancer and noncancer pain. *Curr Pain Headache Rep* 2001; 5:241-249.
6. Schug SA, Saunders D, Kurowski I, Paech MJ. Neuraxial drug administration: a review of treatment options for anaesthesia and analgesia. *CNS Drugs* 2006; 20:917-933.
7. Hayek SM, Deer TR, Pope JE, Panchal SJ, Patel VB. Intrathecal therapy for cancer and non-cancer pain. *Pain Physician* 2011; 14:219-248.
8. Harned ME, Salles SS, Grider JS. An introduction to trialing intrathecal baclofen in patients with hemiparetic spasticity: A description of 3 cases. *Pain Physician* 2011; 14:483-489.
9. Lima RM, Navarro LH, Carness JM, Barros GA, Marques ME, Solanki D, Ganem EM.Clinical and histological effects of the intrathecal administration of methylprednisolone in dogs. *Pain Physician* 2010; 13:493-501.
10. Dureja GP, Usmani H, Khan MM, Tahseen M, Jamal A. Efficacy of intrathecal midazolam with or without epidural methylprednisolone for management of post-herpetic neuralgia involving lumbosacral dermatomes. *Pain Physician* 2010; 13:231-221.
11. Grider JS, Harned ME, Etscheidt MA. Patient selection and outcomes using a low-dose intrathecal opioid trialing method for chronic nonmalignant pain. *Pain Physician* 2011; 14:343-351.
12. Deer TR, Smith HS, Burton AW, Pope JE, Doleys DM, Levy RM, Staats PS, Wallace MS, Webster LR, Rauck RL, Cousins M. Comprehensive consensus based guidelines on intrathecal drug delivery systems in the treatment of pain caused by cancer pain. *Pain Physician* 2011; 14:E283-E312.
13. Raffaeli W, Sarti D, Demartini L, Sotgiu A, Bonezzi C; Italian Ziconotide Group. Italian registry on long-term intrathecal ziconotide treatment. *Pain Physician* 2011; 14:15-24.
14. Deer TR, Smith HS, Cousins M, Doleys DM, Levy RM, Rathmell JP, Staats PS, Wallace M, Webster LR. Consensus guidelines for the selection and implantation of patients with noncancer pain for intrathecal drug delivery. *Pain Physician* 2010; 13:E175-213.
15. Yaksh TL, Grafe MR, Malkmus S, Rathbun ML, Eisenach JC. Studies on the safety of chronically administered intrathecal neostigmine methylsulfate in rats and dogs. *Anesthesiology* 1995; 82:412-427.
16. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 17:1031-1036
17. Kawakami M, Weinstein JN, Spratt KF, Chatani K, Traub RJ, Meller ST, Gebhart GF. Experimental lumbar radiculopathy. Immunohistochemical and quantitative demonstrations of pain induced by lumbar nerve root irritation of the rat. *Spine (Phila Pa 1976)* 1994; 19:1780-1794.
18. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994; 53:55-63.
19. Hope-Simpson RE. The nature of herpes zoster: A long-term study and a new hypothesis. *Proc R Soc Med* 1965; 58:9-20.
20. Argoff CE, Katz N, Backonja M. Treatment of postherpetic neuralgia: A review of therapeutic options. *J Pain Symptom Manage* 2004; 28:396-411.
21. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ. Neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 2000; 342:635-645.
22. Amlie-Lefond C, Kleinschmidt-DeMasters BK, Mahalingam R, Davis LE, Gilden DH. The vasculopathy of varicella-zoster virus encephalitis. *Ann Neurol* 1995; 37:784-790.
23. Kleinschmidt-DeMasters BK, Gilden DH. Varicella-Zoster virus infections of the nervous system: Clinical and pathologic correlates. *Arch Pathol Lab Med* 2001; 125:770-780.
24. Oaklander AL. Mechanisms of pain and itch caused by herpes zoster (shingles). *J Pain* 2008; 9:S10-18.
25. Decroix J, Partsch H, Gonzalez R, Mobacken H, Goh CL, Walsh L, Shukla S, Naisbett B. Factors influencing pain outcome in herpes zoster: An observational study with valaciclovir. Valaciclovir International Zoster Assessment

Group (VIZA). *J Eur Acad Dermatol Venereol* 2000; 14:23-33.

26. McKendrick MW, McGill JI, White JE, Wood MJ. Oral acyclovir in acute herpes zoster. *Br Med J (Clin Res Ed)* 1986; 293:1529-1532.
27. Morton P, Thomson AN. Oral acyclovir in the treatment of herpes zoster in general practice. *N Z Med J* 1989; 102\:93-95.
28. Wood MJ, Shukla S, Fiddian AP, Crooks RJ. Treatment of acute herpes zoster: Effect of early (< 48 h) versus late (48-72 h) therapy with acyclovir and valaciclovir on prolonged pain. *J Infect Dis* 1998; 178:S81-84.
29. Huff JC, Bean B, Balfour HH, Jr., Laskin OL, Connor JD, Corey L, Bryson YJ, McGuirt P. Therapy of herpes zoster with oral acyclovir. *Am J Med* 1988; 85:84-89.
30. Elion GB. The biochemistry and mechanism of action of acyclovir. *J Antimicrob Chemother* 1983; 12 :9-17.
31. Elion GB, Furman PA, Fyfe JA, de Miranda P, Beauchamp L, Schaeffer HJ. The selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Reproduced from Proc. Natl. Acad. Sci. USA 74, 5716-5720 (1977). *Rev Med Virol* 1999; 9:147-152; discussion 152-143.
32. Lycke J, Andersen O, Svennerholm B, Appelgren L, Dahlof C. Acyclovir concentrations in serum and cerebrospinal fluid at steady state. *J Antimicrob Chemother* 1989; 24:947-954.
33. Coda BA, Brown MC, Schaffer R, Donaldson G, Jacobson R, Hautman B, Shen DD. Pharmacology of epidural fentanyl, alfentanil, and sufentanil in volunteers. *Anesthesiology* 1994; 81:1149-1161.
34. Adair JC, Gold M, Bond RE. Acyclovir neurotoxicity: Clinical experience and review of the literature. *SouthMed J* 1994; 87(:1227-1231.
35. Becker BN, Schulman G. Nephrotoxicity of antiviral therapies. *Curr Opin Nephrol Hypertens* 1996; 5:375-379.
36. Haefeli WE, Schoenenberger RA, Weiss P, Ritz RF. Acyclovir-induced neurotoxicity: Concentration-side effect relationship in acyclovir overdose. *Am J Med* 1993; 94:212-215.
37. Johnson GL, Limon L, Trikha G, Wall H. Acute renal failure and neurotoxicity following oral acyclovir. *Ann Pharmacother* 1994; 28:460-463.
38. Revankar SG, Applegate AL, Markovitz DM. Delirium associated with acyclovir treatment in a patient with renal failure. *Clin Infect Dis* 1995; 21:435-436.