NOTE *Laboratory Animal Science*

Usage of Analgesic in a Murine Model Infected Latently with Pseudorabies Virus

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ABSTRACT. Butorphanol tartrate (BT) was injected into mice before injection with acetylcholine in a murine model infected latently with pseudorabies virus. The analgesic effect and its influence on virus reactivation were observed. Mice preinjected with BT showed suppression of screaming, moving and excitation and the same level of movement after excitation as mice injected with PBS. In the group injected with BT i.p., one mouse died and another developed diarrhea with increased virus excretion. These results showed that BT has analgesic effects by both injection routes, s.c. and i.p.; however, BT induced death as a side effect, especially with i.p. injection. The injection route for BT should therefore be investigated further.

KEY WORDS: analgesic, butorphanol tartrate, latent infection, murine model, Pseudorabies virus.

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The establishment of latent infections is an extremely interesting feature of all Herpes viruses. The viruses of alphaherpesvirinae, one of the 3 subfamilies of *herpesviridae*, persist in an inactive state, primarily in neural tissues and mainly within the neurons of ganglia, for varying durations [1, 24] and avoid the host's immune responses. Such latent viruses are often reactivated by stress [14, 15, 23]. Herpes virus infection thus results in a long-term course of recurrent disease. Animal models provide experimental systems for elucidating the molecular mechanisms of latent viral reactivation.

Pseudorabies virus (PrV), a member of the *alphaherpesvirindae* subfamily, causes Aujezskey's disease (AD). Piglets infected with PrV die of acute symptoms within a few days. In contrast, the clinical signs in adults include coughing, sneezing, lethargy, nervousness, uncoordinated movements and abortion of infected pregnant sows. The virus becomes localized in the trigeminal ganglia of infected pigs and establishes a latent infection there [2, 3, 18, 20]. Latently infecting viruses may be reactivated by stress, such as transportation, change of food and/or several diseases [29, 32]. Complete clearance of AD is difficult once it invades a farm, since it is almost impossible to distinguish latently infected pigs based on uninfected pigs from their outward appearance.

We previously reported that latent PrV infection in swine could be reactivated by treatment with acetylcholine both *in vivo* and *in vitro* [25]. We have also established a PrV latent infection model in mice with the wild PrV YS-81 strain [26]. The mice were pretreated with anti-PrV swine serum and then challenged with YS-81 based on a procedure reported by Osorio and Rock [16]. Almost all the mice survived, and PrV was detected and reactivated in the trigeminal ganglia (TGs) of the mice. PrV was reactivated in latently infected mice *in vivo* by stimulation with acetylcholine or dexamethasone [27]. Thus, we established a procedure for reactivating latent PrV with acetylcholine.

Injection of acetylcholine to reactivate a latent virus causes transient stress to mice, which can reactivate the latent virus; however, this means the mice are able to avoid stress using this system. If we can achieve comparable results by decreasing the load on mice, this will improve the experiment and animal welfare.

When considering animal welfare in experiments, anesthesia, sedation and analgesia are important factors. Recently, anesthesia and sedation have been utilized in many kinds of animal experiments [5]; however, analgesia is rarely discussed in this field, especially for small animals such as rodents.

In this research, butorphanol tartrate (BT), an analgesic for dogs and cats [17, 31], was injected into mice before latent virus reactivation by acetylcholine injection, and the analgesic effect and influence on virus reactivation were observed.

BALB/c mice were purchased from Charles River Laoratory Japan, Inc. and used for latent infection. The animal experiments were approved by the Committee on Animal Experiments of Oita University and undertaken in accordance with the Guidelines for Animal Experimentation, Oita University. Acetylcholine chloride (ACH) was purchased from Wako Pure Chemical Industries, Ltd. BT was purchased from Meiji, Japan. PrV wild strain YS-81 was grown in porcine kidney cells (PK-15), and the virus titer was assayed in cloned PK cells (CPK) [7]. Cells were grown in Eagle's minimum essential medium (MEM) containing 5% fetal bovine serum, 1.5% $NaHCO_3$ and 0.1% each of penicillin G potassium, streptomycin sulphate and kanamycin sulphate.

Five-week-old mice were passively immunized by intraperitoneal (i.p.) inoculation of 0.25 m*l* anti-PrV swine serum. The final neutralization titer of this serum was 1:128. Thirty minutes later, preimmunized animals were infected with 100 lethal dose 50s (LD_{50}) of YS-81 i.p. Mice that survived the challenge were kept for 2 months and used

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as latently infected (LI) mice. The presence of latent PrV DNA was assessed in the TGs of these LI mice by polymerase chain reaction (PCR) amplification of a 531-bp target sequence contained in the gene encoding PrV gG, according to the method described in our previous report [26], after the mice had been euthanized. LI mice were inoculated i.p. or subcutaneous (s.c.) with 0.2, 0.4 or 0.8 mg/kg of BT. For the control group, phosphate-buffered saline (PBS) was inoculated i.p. One hour later, the latent virus was stimulated by inoculation with 2.73 mg/mouse ACH i.p. dissolved in 0.5 m*l* PBS. At inoculation, screaming, twisting of the body, excitability and depression after excitability were observed when evaluating the analgesic effect of BT. Only screaming time was recorded because it was facilely counted. Nasal swab specimens were taken daily for detection of virus excretion. Under anesthesia, 100 μl MEM was injected into one of the nasal cavities of each LI mouse. The wash was taken from the other cavity and the mouth with a swab (men-tip, J.C.B Industry Limited, Tokyo, Japan) as a nasal swab sample. The swab specimen was immersed in 300 μl MEM and stored at –80°C until the virus isolation test. The presence of latent PrV DNA was assessed in nasal swab specimens by PCR amplification of a 531-bp target sequence contained in the gene encoding PrV gG, following the method described in our previous report [28]. DNA was extracted from the swab specimens with ISOGEN-LS (Wako Pure Chemical, Osaka, Japan). The TGs or brain was minced with a cell strainer (Falcon, BD, Franklin Lakes, NJ, U.S.A.), and the DNA was extracted with ISOGEN (Wako Pure Chemical Industries, Ltd.). The DNA samples and oligonucleotide primers were initially heated at 94°C for 2 min, denatured at 94°C for 1 min, annealed at 56°C for 1 min and extended at 72°C for 2 min. The samples were then subjected to 30 cycles of amplification and maintained at 72°C for 7 min. The sequence of the forward primer was AGCGGTAGGACACACACACC, and that of the reverse primer was AGACGAGCACGACGATGTAC. Amplification products were analyzed by electrophoresis on 1.0% agarose gels. The statistical analysis of the data was performed by Student's *t*-test for screaming time, and the chi-square test was used for other data.

Thirty-six LI mice were analyzed. Some regions of TGs were harvested separately, and DNA was purified for detection of PrV by PCR. PrV DNA was detected, and latent infection was confirmed in all mice (data not shown).

To investigate the analgesic effects of BT, LI mice were inoculated i.p. or s.c. with several concentrations of BT prior to ACH stimulation. As shown in Table 1, mice injected with BT showed decreased screaming, twisting of the body and excitability when stimulated with ACH compared with the PBS control group; in particular, s.c. injection showed a higher analgesic effect than i.p., although there was little difference in the effect on depression after excitability by both routes. In the i.p. injection group, one

Table 1. Analgesic effect of BT in ACH stimulation

Injection route	BT conc.	Mice	Screaming			Twisting of the body		Excitability		Depression	Notes
			Times	Mice	%	Mice	%	Mice	%		
I.p.	0.8 mg/kg	5	1.2	3 *	60	3	60	0 ***	0	5	1 diarrhea
	0.4 mg/kg	5	1.2	3 *	60	4	80	0 ***	0	5	1 died on day 1
	0.2 mg/kg	5	3.2	4	80	3	60	3 *	60	5	1 diarrhea
S.c.	0.8 mg/kg	5	0 **	0 ***	0	0 ***	0	0 ***	0	5	
	0.4 mg/kg	5	0 **	0 ***	0	0 ***	0	0 ***	0	5	
	0.2 mg/kg	5	0 **	0 ***	0	2	40	0 ***	0	5	
	Control	6	2.8	6	100	5	83	6	100	6	1 died on day 3

LI mice were inoculated with BT intraperitoneally or subcutaneously. One hour later, mice were stimulated with intraperitoneal injection of ACH, and the levels of screaming, twisting of the body, excitability and depression were observed. Data are shown as the number of mice showing each reaction and the percentage (%). The average screaming time is also shown. The significance compared with the control group is indicated as follows: *, p<10%; **, p<5%; ***, p<1%.

Table 2. Virus reactivation at ACH stimulation of LI mice pretreated with BT

Injection route	BT	Mice	Day 0	Day 1	Day 2	Day 3
I.p.	0.8 mg/kg	5	0	2	3	3
	0.4 mg/kg	5	0	3	3	2
	0.2 mg/kg	5	0	3	4	2
S.c.	0.8 mg/kg	5	0	1	0	0*
	0.4 mg/kg	5	0	3	1	0
	0.2 mg/kg	5	0	0	2	3
	Control	6	0	1	3	1

Swab samples were taken after stimulation with ACH from LI mice pretreated with BT. The data represent the number of LI mice showing a positive reactivation for PrV by PCR. Significance compared with the control group is indicated by an asterisk (*: p<10%).

mouse in each group injected with serially diluted BT developed diarrhea, and one mouse died during the test period. None of the other groups showed any side effects.

To verify whether BT influences virus reactivation with ACH, virus excretion was analyzed, and the results are shown in Table 2. Virus excretion in nasal swabs was confirmed in all groups, and excretion in the group injected i.p. with BT was higher than in the s.c. injection group and PBS control group.

Maintenance of satisfactory animal welfare is mandatory, and narcotic drugs are generally utilized in animal experiments [5]. As a further step, analgesia is also starting to be utilized; however, it is currently limited to mid-sized animals, such as dogs and cats [11], and there is little usage in experiments utilizing small animals, such as rodents.

The analgesic effect of BT has been reported in dogs [4, 21], cats [10, 12], ponies [13] and ferrets [22]. In small animals, rabbits [30], rats [8] and mice [6, 8, 9], BT has sufficient analgesic effects. Today, BT is available commercially as an analgesic for dogs and cats; however, its use is not permitted in rodents. In our experimental murine model, BT had a sufficient analgesic effect, especially when inoculated s.c. Our results support the increased range of BT usage.

We utilized i.p. inoculation to administer BT because other chemicals are practically inoculated i.p. in our infection experimental model system. When constructing an experimental model, it is advisable to simplify the protocol. However, some mice inoculated with BT i.p. showed intragastric problems. Roebel *et al.* reported that intravenous injection of BT had little or no effect on the biliary or gastrointestinal system in dogs [19]. It is important to consider side effects in different species or inoculation routes when using BT as an analgesic.

BT showed analgesic effects in the murine latent infection model of PrV, especially by s.c. injection. Because BT infection by both routes did not decrease virus reactivation, BT is useful as analgesic in this model. It is possible that injection of BT i.p. increases the reactivation of latent PrV. Injection i.p. had excessive side effects, whereas injection s.c. showed comparable virus excretion to the previous method without any side effects. These side effects may have a synergistic effect with ACH and increase reactivation of a latent virus.

In conclusion, it was shown that BT has analgesic effects by both the s.c. and i.p. injection routes; however, BT induced death as a side effect, especially with i.p. injection. The injection route of BT should therefore be investigated further.

REFERENCES

1. Ahmed, R. and Stevens, J. G. 1990. Viral persistence, pp. 241–266. *In*: Virology (Fields, B. N. and Knipe, D. M. eds.), Raven Press, New York.
2. Ben-Porat, T., Deatly, A. M., Easterday, B. C., Galloway, D., Kaplan, A. S. and McGregor, S. 1984. Latency of pseudorabies virus. *Curr. Top. Vet. Med. Anim. Sci.* **27**: 365–383.
3. Cheung, A. K. 1989. Detection of pseudorabies virus transcripts in trigeminal ganglia of latent infected swine. *J. Virol.* **63**: 2908–2913.
4. Christie, G. J., Strom, P. W. and Rourke, J. E. 1980. Butorphanol tartrate: a new antitussive agent for use in dogs. *Vet. Med. Small Anim. Clin.* **75**:1559–1562.
5. Donovan, J. and Brown, P. 2001. Anesthesia. *Curr. Protoc. Immunol.* Chapter 1:Unit 1.4.
6. Filibeck, U., Castellano, C. and Oliverio, A. 1981. Differential effects of opiate agonists-antagonists on morphine-induced hyperexcitability and analgesia in mice. *Psychopharmacology* (*Berl.*) **73**:134–136.
7. Fukusho, A., Ogawa, N., Yamamoto, H., Sawada, M. and Sazawa, H. 1976. Reverse plaque formation by hog cholera virus of the GPE-strain inducing heterologous interference. *Infect. Immunol.* **14**: 332–336.
8. Gades, N. M., Danneman, P. J., Wixson, S. K. and Tolley, E. A. 2000. The magnitude and duration of the analgesic effect of morphine, butorphanol, and buprenorphine in rats and mice. *Contemp. Top. Lab. Anim. Sci.* **39**:8–13.
9. Garner, H. R., Burke, T. F., Lawhorn, C. D., Stoner, J. M. and Wessinger, W. D. 1997. Butorphanol-mediated antinociception in mice: partial agonist effects and mu receptor involvement. *J. Pharmacol. Exp. Ther.* **282**:1253–1261.
10. Gross, M. E., Smith, J. A. and Tranquilli, W. J. 1993. Cardiorespiratory effects of combined midazolam and butorphanol in isoflurane-anesthetized cats. *Vet. Surg.* **22**:159–162.
11. Hansen, B. and Hardie, E. 1993. Prescription and use of analgesics in dogs and cats in a veterinary teaching hospital: 258 cases (1983–1989) *J. Am. Vet. Med. Assoc.* **202**:1485–1494.
12. Hosgood, G. 1990. Pharmacologic features of butorphanol in dogs and cats. *J. Am. Vet. Med. Assoc.* **196**:135–136.
13. Kalpravidh, M., Lumb, W. V., Wright, M. and Heath, R. B. 1984. Effects of butorphanol, flunixin, levorphanol, morphine, and xylazine in ponies. *Am. J. Vet. Res.* **45**:217–223.
14. Martin, J. R. and Suzuki, S. 1987. Inflammatory sensory polyradiculopathy and reactivated peripheral nervous system infection in a genital herpes model. *J. Neurol. Sci.* **79**: 155–171.
15. Openshaw, H., Ahavrina, L. V., Wohlenberg, C., Sekizawa, T. and Notkins, A. L. 1979. Acute and latent infection of sensory ganglia with herpes simplex virus: Immune control and reactivation. *J. Gen. Virol.* **44**: 205–215.
16. Osorio, F. A. and Rock, D. L. 1992. A murine model of pseudorabies virus latency. *Microbiol. Pathol.* **12**: 39–46.
17. Peter, W. H. 1999. Minimizing postoperative disconfort in dogs and cats. *Vet. Med.* 259–265.
18. Priola, S. A., Gustafson, D. P., Wagner, E. K. and Stevens, J. G. 1990. A major portion of the latent pseudorabies virus genome is transcribed in trigeminal ganglia of pigs. *J. Virol.* **64**: 4755–4760.
19. Roebel, L. E., Cavanagh, R. L. and Buyniski, J. P. 1979. Comparative gastrointestinal and biliary tract effects of morphine and butorphanol (Stadol). *J. Med.* **10**:225–238.
20. Rziha, H. J., Mettenleiter, T. C., Ohlinger. V. and Wittmann, G. 1986. Herpesvirus (pseudorabies virus) latency in swine: occurrence and physical state of viral DNA in neural tissues. *Virology* **155**: 600–613.
21. Schurig, J. E., Cavanagh, R. L. and Buyniski, J. P. 1978. Effect of butorphanol and morphine on pulmonary mechanics, arterial blood pressure and venous plasma histamine in the anesthetized dog. *Arch. Int. Pharmacodyn. Ther.* **233**: 296–304.
22. Schurig, J. E., Florczyk, A. P., Rose, W. C. and Bradner, W. T.

1982. Antiemetic activity of butorphanol against cisplatin-induced emesis in ferrets and dogs. *Cancer Treat. Rep.* **66**: 1831–1835.
23. Shimeld, C., Hill, T. J., Blyth, W. A. and Easy, D. L. 1990. Reactivation of latent infection and induction of recurrent herpes eye disease in mice. *J. Gen. Virol.* **71**: 397–404.
24. Stevens, J. G. 1989. Human herpesviruses; a consideration of the latent state. *Microbiol. Rev.* **53**: 318–322.
25. Tanaka, S., Imamura, T., Sakaguchi, M., Mannen, K. and Matsuo, K. 1996. Acetylcholine activates latent pseudorabies virus in pigs. *Arch. Virol.* **141**: 161–166.
26. Tanaka, S., Imamura, T., Sakaguchi, M. and Mannen, K. 1998. Acetylcholine reactivates latent pseudorabies virus in mice. *J. Virol. Methods* **70**: 103–106.
27. Tanaka, S. and Mannen, K. 2002. Activation of Latent Pseudorabies Virus Infection in Mice Treated with Acetylcholine. *Exp. Anim.* **51**: 407–409.
28. Tanaka, S. and Mannen, K. 2004. Role of IL-1β in reactivation by acetylcholine of latently infecting pseudorabies virus. *Exp. Anim.* **53**: 457–461.
29. Thawley, D. G., Solorzano, R. J. and Johnson, M. E. 1984. Confirmation of pseudorabies virus infection, using virus recrudescence by dexamethasone treatment and *in vitro* lymphocyte stimulation. *Am. J. Vet. Res.* **45**: 981–983.
30. Turner, P. V., Kerr, C. L., Healy, A. J. and Taylor, W. M. 2006. Effect of meloxicam and butorphanol on minimum alveolar concentration of isoflurane in rabbits. *Am. J. Vet. Res.* **67**: 770–774.
31. Wagner, A. E. 1999. Is butorphanol analgesic in dogs and cats? *Vet. Med.* **94**: 346–351.
32. Wittmann, G., Ohlinger, V. and Rziha, H. J. 1983. Occurrence and reactivation of latent Aujeszky's disease virus following challenge in previously vaccinated pigs. *Arch. Virol.* **75**: 29–41.