Safety and efficacy of buprenorphine for analgesia in laboratory mice and rats

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Buprenorphine is a long-acting opiate with a high therapeutic index. The authors review the pharmacology, toxicity, analgesic effects and delivery of buprenorphine for use in laboratory mice and rats. Buprenorphine-based analgesic therapy has a substantial record of safety, and there is growing evidence of its effectiveness for treating post-operative pain. Nonetheless, more research is needed to determine optimal delivery systems and analgesic regimens for pain therapy in laboratory animals.

Buprenorphine was invented in the late 1960s by John Lewis and Alan Cowan, who were synthesizing new analgesics based on the structure of morphine. Several compounds showed promise in animal studies, including one in particular: buprenorphine[1]. Safety studies in animals have shown that the drug has an unusually high therapeutic index[2]: the ratio of the lethal dose (LD50) to the effective concentration (EC50) is at least three times greater than that of morphine, which is considered a safe analgesic for animals and humans[3].

Buprenorphine use in humans was curtailed in the late 1980s in response to numerous case reports describing deaths associated with intravenous (i.v.) overdoses. Over the next decade, follow-up toxicology studies showed that in almost every case, the victim had mixed buprenorphine with lethal combinations of other drugs including heroin, methadone and benzodiazepines[4].

Following reports of low toxicity and a high affinity for opioid receptors, the drug has been increasingly accepted in veterinary medicine for analgesic use. Sold under the name Vetergesic (Alstoe Ltd, York, UK), buprenorphine has been described as an excellent drug for use in companion animals[5,6]. Cowan and others have advocated the drug's use in laboratory animals[7–9], given that growing regulatory considerations require effective and humane postoperative analgesia for all animals involved in experimental surgery[10,11].

Toxicity studies coupled with an understanding of pharmacology are keys to drug safety. In this article, we review the pharmacology, toxicology, analgesic effects and delivery methods of buprenorphine for use in laboratory animal studies.

PHARMACOLOGY

Buprenorphine is an opioid with unique pharmacology. It can act as an agonist and antagonist at different classes of opioid receptors. Chronic spinal injury studies in dogs have confirmed the drug's action as an agonist at the μ-opioid receptor[12]. Subsequent studies showed that buprenorphine can also bind to κ- and δ-opioid receptors and block ε-opioid receptors[13]. Studies with knockout mice have shown that the antinociceptive effect of buprenorphine, which is mediated primarily by the μ-opioid receptor, is attenuated by the ability of the drug to activate the opioid receptor–like (ORL-1) receptor[14]. The drug has been described as both a full agonist and a partial agonist at the same receptor, depending on the assay used[15].

Agonism at the μ-opioid receptor and, in some cases, antagonism at the κ- or δ-opioid receptors are possible underlying mechanisms for the ceiling effect and bell-shaped dose-response curve of buprenorphine. There appears to be no ceiling effect for buprenorphine's analgesic properties. There is a ceiling effect for the respiratory depression induced by buprenorphine, reducing the likelihood of this potentially fatal adverse event[16].

Pharmacokinetic studies with bolus injections of buprenorphine show similar results in mice, rats and humans. After bolus i.v. administration, the drug is N-dealkylated in the liver to the active metabolite norbuprenorphine (NBN) and plasma levels decline

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tri-exponentially[17]. The plasma half-lives of buprenorphine and NBN are ~5–7 h in the rat[18]. In one study of rats, the amounts of un-metabolized buprenorphine excreted in the urine and feces 1 week after injection were 1.9% and 22.4% of the dose, respectively; 92% of the dose was excreted or metabolized within 1 week[19]. Similar excretion data was reported for mice[20]. Glucuronide metabolites of buprenorphine and NBN are also metabolically active and can approximate or exceed the plasma concentrations of the parent drug[21].

TOXICOLOGY

Early reviews of buprenorphine indicated that the new drug provided few advantages over other opiates: its oral bioavailability was inferior to morphine, and it appeared to produce side effects, like respiratory depression, similar to those seen with other morphine-like compounds[22]. But a wide range of *in vitro* and *in vivo* studies have subsequently shown that buprenorphine is substantially less toxic than other opiates.

Toxicity

Because buprenorphine is metabolized in the liver, *in vitro* studies have been done to examine its effects on hepatocytes, liver mitochondria and cytochrome P_{450} systems. The *in vitro* toxicity risk index of buprenorphine, like that of meperidine, is between those of morphine and methadone[23]. Buprenorphine and NBN undergo cytochrome P_{450} 3A–mediated covalent binding to rat liver microsomal proteins, and both cause moderate glutathione depletion, suggesting that the hepatotoxicity seen in hepatitis patients with buprenorphine overdoses is mainly the result of mitochondrial effects[24].

Efforts to gauge the effects of buprenorphine on normal and abnormal nerve tissue have produced conflicting results. Buprenorphine induces dose-dependent apoptosis in mouse neuroblastoma × rat glioma hybrid NG108-15 cells, and a time-course experiment indicated that DNA fragmentation occurs in the cells within 4 h after buprenorphine administration[25].

The effects of intraperitoneal (i.p.) buprenorphine (0.05 mg per kg body weight) in Sprague Dawley rats after collagenase-induced intracerebral hemorrhage have been investigated[26]. Although the hematoma reduced in volume, the number of necrotic neurons in the penumbra was significantly increased. These neurological and histopathological changes were assumed to be the result of administration of buprenorphine, but this conclusion should be interpreted carefully before being applied to the use of opioid analgesics in similar models. In a transient model of focal cerebral ischemia in rats, an acute dose of 2 mg buprenorphine per kg body weight was administered during the postoperative period. The absence of additional toxicity provided preclinical support for the safety of buprenorphine use after an ischemic event, as well as for maintenance therapy of opioid-dependent patients, for whom the risk of cerebrovascular hemorrhage is increased[27]. In another study with rats, orally supplied buprenorphine had no apparent adverse effects on an ischemia model and appeared to minimize stress-associated confounding variables[28].

Buprenorphine has been safely used in a rat model of spinal cord ischemia as well. Neither pentazocine (150 μg) nor buprenorphine (4 μg) produced neurological dysfunction, in contrast with intrathecal morphine (30 μg), which induced spastic paraparesis after 6 min of aortic occlusion[29].

As for peripheral nervous system effects, sustained exposure to opiates including buprenorphine can lead to hyperalgesia, apparently owing to an NMDA-receptor mechanism. The nociceptive effects of buprenorphine appear to be bimodal: it is pronociceptive at ultra-low doses and antinociceptive at higher doses[30].

Prenatal and neonatal safety

The results of a survey indicating an opioid dependence rate of about 15% in pregnant women[31] has led to investigations of the effects of opiates on pregnancy and placental metabolism. Maternally administered buprenorphine in rats crosses the placenta and is present in mother's milk[32]. Developmental effects on brain neurotransmitters and their receptors vary depending on the dose, route of administration, duration of drug exposure and the age of the pups when evaluated[32]. Buprenorphine appears to have fewer offspring effects than methadone[32].

Studies evaluating the safety of single-dose buprenorphine treatment of mice undergoing embryo transfer indicate that the number of viable implanted embryos is unaffected by the treatment[33,34]. There are conflicting reports on the effects of chronic prenatal exposure to buprenorphine. When rats were continuously from gestational day 8 through parturition using osmotic minipumps, a dose-dependent reduction in maternal water intake was observed, but no effects were observed on maternal weight gain, the frequency of resorptions, perinatal mortality or birth weight, and inconsistent effects on postnatal growth were observed[35]. In contrast, other investigators found that, although perinatal exposure to buprenorphine failed to produce severe maternal, prenatal or neonatal mortality, it was associated with a significant increase in perinatal mortality and perturbations of pup development[36–38].

Hematology and immune effects

The effects of chronic buprenorphine exposure on hematological parameters have also been studied. A severe decrease in white blood cells and hematocrit value was noted in Swiss mice injected with 0.3 mg of i.p. buprenorphine daily for 60 consecutive d (ref. 39). These abnormalities reverted to normal within 45 d

after withdrawal of the drug. Oral buprenorphine had anti-inflammatory activity in a rat model of streptococcal cell wall–induced arthritis[40]. In a bone slice assay, buprenorphine inhibited osteoclastic bone resorption with a half-maximal inhibitory concentration (IC50) of 1 μM (ref. 41). The same study found that buprenorphine exacerbated inflammation in a rat adjuvant arthritis model *in vitro*, suggesting that attempts to use buprenorphine for analgesia in this model may lead to complications.

Daily subcutaneous (s.c.) buprenorphine injections for 4 weeks in rats were found to have only modest effects on the immune system[42]. To characterize the effects of postsurgical opioid use in a cecal ligation and puncture model of sepsis, female ICR mice were administered 80 mg tramadol, 20 mg tramadol or 0.1 mg buprenorphine per kg body weight for 3 weeks. Although there were no differences between the groups in cell count, differentials and cytokine levels in blood, peritoneum and airways, tramadol treatment resulted in more and later deaths than treatment with buprenorphine[43].

Subsequent studies in animal models of infectious diseases and autoimmune disorders have concluded that, unlike morphine and fentanyl, there is no known immunosuppressive activity with buprenorphine at therapeutic dose levels[44–48].

Respiratory depression

Buprenorphine has been reported to cause respiratory depression, a common effect with the use of opioid analgesics[22]. When rats were injected i.v. with a potentially lethal dose of buprenorphine (90 mg per kg body weight), the dose had no effect on arterial blood gases in surviving rats[49]. In another study of the effects of i.p. administration of four opioids, including buprenorphine, on arterial blood gases and plethysmography to identify opioid molecule–specific patterns and classify response severity, all drugs increased inspiratory time at 10% to 80% of their LD50 (ref. 50). In this and another study[51], buprenorphine caused only hypoxemia, whereas morphine also caused hypercapnia and methadone and fentanyl induced hypoxemia, hypercapnia and expiratory time increases.

The respiratory depression induced by NBN in rats was found to be more severe than that induced by buprenorphine. After i.v. bolus administration of buprenorphine in rats, no changes in respiratory rate and arterial carbon dioxide partial pressure (pCO_2) levels were noted over the dose range 0.008–3 mg per kg body weight. In contrast, after rapid i.v. administration of NBN, the respiratory rate decreased in a dose-dependent fashion within the dose range of 1–3 mg per kg body weight. Arterial pCO_2 levels also varied in proportion to the change in respiratory rate. The minimum respiratory rate was observed 15 min after NBN administration. Judging by this respiratory depressive effect, NBN is approximately 10 times more potent than its parent drug[52]. A simulation showed, however, that the concentrations of NBN following i.v. administration of buprenorphine reach values well below the values shown to effect respiration[53].

ANALGESIA FOR LABORATORY ANIMALS

Attempts to visually score pain in rats and mice given analgesics can be difficult because analgesics may have side effects that inadvertently affect the score[54]. Generally, measuring the time to withdraw a paw or tail from a heat stimulus has been an accepted method of assaying the analgesic effect. Another method is the writhing assay, in which an irritant is injected i.p. and subsequent writhing movements are counted.

Numerous clinical studies have evaluated the efficacy of various drug-plasma concentrations in humans. Plasma drug levels greater than 0.5 ng/ml have been associated with pain relief in at least 50% of trial populations, and values greater than 1 ng/ml were associated with pain relief in a majority of clinical reports[55,56]. Investigators therefore have relied on the human metric of ~1 ng/ml of plasma buprenorphine to indicate clinically relevant blood levels in animals including rodents. The analgesic efficacy of buprenorphine at blood levels of 1 ng/ml or more has been verified in rats, as demonstrated by positive results on tail flick latency tests[57] and paw withdrawal latency tests[58].

The analgesic efficacy of buprenorphine has been assessed in several rodent models of acute and chronic pain[59]. Full analgesia was obtained in mouse models of acute pain, in mice and rats after yeast- and formalin-induced inflammatory pain and in mice and rats after mustard oil–induced hyperalgesia. The authors concluded that buprenorphine shows a broad analgesic profile and offers a means of treating multiple pain conditions, including neuropathic pain.

Diabetic peripheral neuropathy can induce loss of nociception as well as mechanical hyperalgesia and tactile allodynia. In Sprague Dawley male rats, buprenorphine infusions from osmotic minipumps significantly reversed the diabetes-induced allodynia for up to 7 d of treatment without altering thermal perception or nerve conduction velocity[60].

In a study of postoperative analgesia for partial hepatectomy in C57BL/6NCrl male mice, meloxicam and buprenorphine (0.1 mg per kg body weight, twice daily for 3 d) were effective, whereas the nonsteroidal anti-inflammatory drug flunixin meglumine (2.5 mg per kg body weight) did not appear to provide adequate analgesia[61].

The effectiveness of buprenorphine for the relief of pain induced by intestinal resection is still under investigation[62]. When body weight changes are used as a surrogate marker for pain relief, meloxicam may be a better choice for analgesia compared to buprenorphine[63,64];

oxymorphone was also reported to be more effective than buprenorphine[65]. In other studies, buprenorphine proved more effective than carpofen, ketoprofen and acetaminophen, based on a 'grimace scale' assessment of pain in mice[66], and more effective than tramadol and tramadol-gabapentin[67]. The challenges of comparing methods to monitor recovery in surgically treated rodents have been reviewed[68].

SUSTAINED DELIVERY STRATEGIES

Although literature reports confirm the safety and efficacy of opiates for the treatment of postsurgical pain, questions remain concerning the optimal delivery strategy. Limitations of oral, i.p. and i.v. routes of analgesia administration have been cited[6,69]. Objections to i.p. and s.c. therapy sort along economic and technical concerns: depending on the model, opiate therapy requires injections at intervals of 4–8 h for 2 d or more, a dose schedule that increases laboratory costs and management challenges. Moreover, multiple daily injections cause stress in surgically treated animals, which may account for discordant results in studies showing negative effects of postsurgical analgesia[70–73]. Osmotic pumps have become a standard method for delivering constant levels of drugs to animals for research[74]. The technology allows opiates to be safely infused into laboratory animals for 1 week or more. Pumps, however, must be removed after their delivery cycle, requiring a second incision and further analgesia. Thus, investigators have focused on oral drug delivery, transdermal patches and implantable drug-release scaffolds, including liquid injectable scaffolds, for the administration of postsurgical analgesia to laboratory animals.

Oral delivery

Oral administration of buprenorphine for the treatment of postsurgical pain has not been proven effective. Whether the effect is secondary to tissue injury or the anesthetic, laboratory animals tend to reduce their food and water intake for 2–3 d after surgery. In rats, 0.5 mg buprenorphine per kg body weight administered by oral gavage was less effective in a paw incision model and on Hargreaves tests, which measure hyperalgesia to thermal stimulation, than was 0.05 mg buprenorphine per kg body weight delivered s.c.[75,76]. In injury-free rats, 0.06 mg/ml buprenorphine supplied in drinking water or flavored foods provided positive thermal latency test scores when combined with s.c. injections of 0.1 mg buprenorphine per kg body weight[77]. The analgesic effect of 0.5 mg/ml buprenorphine administered in food gels was insufficient for mice showing clear signs of discomfort from advanced tumors[78].

Voluntarily ingested buprenorphine may be a pre-emptive treatment strategy for postsurgical pain. Buprenorphine delivered to mice and rats in a chocolate hazelnut paste (Nutella) resulted in 24-h serum concentrations of 110 ng/ml, the same as with gavage dosing[79]. Voluntary preoperative ingestion of buprenorphine–Nutella paste significantly improved postoperative behavior and reduced plasma corticosterone levels in permanently catheterized rats[80].

Transdermal opiates

The transdermal delivery systems Butrans (Purdue Pharmaceuticals LP, Stamford, CT) and Transtec (Napp Pharmaceuticals Ltd, Cambridge, UK) have been approved in the US and Europe for the treatment of moderate to severe chronic pain in humans. Buprenorphine blood levels and pain were assessed in mice given transdermal buprenorphine[81]. Plasma levels of 1 ng/ml afforded positive results in the tail flick assay, but levels of 8 ng/ml or more were needed to produce positive results in the writhing assay. A similar tail flick assay result was described by researchers who used a pharmacokinetic-pharmacodynamic model of the drug's release kinetics to account for its analgesic effects[82]. Whether a transdermal approach could be applied for analgesic buprenorphine treatment after laboratory animal surgery remains to be determined.

Implanted or injected opiate-release scaffolds

Drug-release scaffolds are biocompatible and frequently biodegradable mixtures that bind a drug by weak molecular forces. Early forms of these mixtures were cholesterol-based liposomes or carbohydrate or polylactic acid microspheres. The scaffold-bound drug generally is injected s.c. and is slowly released for days or months.

An s.c. implant of a 50-mg cholesterol-triglyceride-buprenorphine pellet containing 10 mg of the drug afforded 9–12 weeks of analgesia with no evidence of drug toxicity in a rat model[83]. Our laboratory has found evidence of safety for a similar cholesterol-triglyceride-buprenorphine scaffold in mice[84]. Buprenorphine esters have been shown to act as pro-drugs when injected intramuscularly in rats to provide sustained release drug therapy at doses of 0.3–3.0 mg per kg body weight[58]. Alternatively, buprenorphine base suspended in sesame and castor oil and injected intramuscularly can provide a dose effect lasting five to six times longer than injections of buprenorphine hydrochloride in saline[58]. Evaluating the efficacy of a proprietary buprenorphine suspension in rats, researchers found that it afforded plasma concentrations of ~1 ng/ml for 3 d (ref. 85).

CONCLUSIONS

Although buprenorphine appears to be a safe and effective analgesic for humans and companion animals, opinions vary regarding the efficacy of buprenorphine compared to other opiates[86]. The studies reviewed here report several strategies that can provide laboratory

animals with effective treatment for postsurgical pain. New delivery systems and methods to assess acute and chronic pain are still needed.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests. Funding to support this work was obtained from the biotechnology and economic development programs of Maryland Technology Development Corporation (TEDCO) and Maryland Industrial Partnerships (MIPS) and from Bamvet Laboratories, Inc. P.Z. is a paid consultant for Bamvet and is supported by a Johns Hopkins Medical Research Fellowship. M.G. holds a significant financial interest in Bamvet.

Received 4 May 2012; accepted 8 June 2012
Published online at http://www.labanimal.com/

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