The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
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*Sedation of western grey kangaroos (*Macropus fuliginosus ocydromus*) with tiletamine-zolazepam*

*C Mayberry**†, *R Bencini*†, *PR Mawson*‡ *and SK Maloney*§

† School of Animal Biology, The University of Western Australia, Stirling Highway, Crawley, WA 6009, Australia
‡ Perth Zoo, 20 Labouchere Road, South Perth, WA 6151, Australia
§ School of Anatomy, Physiology, and Human Biology, The University of Western Australia, Stirling Highway, Crawley, WA 6009, Australia
* Contact for correspondence and requests for reprints: c.mayberry@ymail.com

Abstract

*It is sometimes necessary to restrain kangaroos (*Macropus *spp) for veterinary treatment or in the course of scientific research, but the associated stresses may induce capture myopathy in wild kangaroos. Judicious use of injectable sedatives can reduce the risk of capture myopathy. Zoletil®, a proprietary mixture of tiletamine and zolazepam, is reported to have a wide safety margin, a small dose volume, and be quick acting for a range of animals. We investigated the dose-response relationship of Zoletil® in 26 western grey kangaroos (*Macropus fuliginosus ocydromus*). All kangaroos were recumbent within 5–10 min of intramuscular injection with mean (± SD) Zoletil® of 4.55 (± 0.98) mg kg^{-1}. Mean (± SD) time to recovery varied between individuals, 2.07 (± 0.41) h over all occasions, and was independent of dose rate. For animals that were assessed on multiple occasions, mean (± SEM) time to recover was reduced from 2.25 (± 0.09) h on the first occasion to 2.15 (± 0.10) h on the second occasion and 1.81 (± 0.11) h on the third. Since kangaroos sedated with Zoletil® are vulnerable to predation and injury during recovery, we believe they should be supervised until they are able to fend for themselves.*

Keywords: *animal welfare, dose-response, recovery, sedation, tiletamine-zolazepam, western grey kangaroos*

Introduction

It is sometimes necessary to restrain kangaroos (*Macropus* spp) for veterinary treatment or in the course of scientific research, and many ways to achieve this have been described. Physical methods include grasping by the tail or netting with a hoop-net, followed by manual restraint or placing the kangaroo in a hessian bag (Poole & Catling 1974); cable-operated 'tunnel traps' that are fixed to gaps where kangaroos pass through or under a fence (Coulson 1996), and cannon nets set at water-points in arid areas (Clancy & Croft 1990). Where stray bullets are not a problem, teams of four people can capture kangaroos at night by dazzling the kangaroos with a bright light and firing a high velocity bullet just over their head (Robertson & Gepp 1982), and fixed traps may be suitable in environments with restricted public access and from which predators have been removed (Algar 1986). Wild kangaroos may also be mustered along a hessian fence into purpose-built yards with walls of hessian (English 2008). These methods can all result in accidental injuries, and strenuous muscle activity as animals panic and/or attempt to escape can precipitate capture myopathy, a debilitating and often fatal condition to which kangaroos are particularly susceptible (Booth 1994). Another method of capturing and restraining kangaroos with a lower attendant risk of capture myopathy is the use of sedatives.

While sedatives reduce the risk of capture myopathy, they are not themselves without risk. The oral administration of sedatives to free-ranging kangaroos by lacing bait or water may affect non-target species and risks over-dosing some individuals while under-dosing others. For instance, the response of western grey kangaroos (*Macropus fuliginosus ocydromus*) to alpha-chloralose in feed or water varied from drowsy and slightly unco-ordinated but able to flee, to comatose, and dead, while surviving kangaroos ignored baits, including water, used a second time (Arnold *et al* 1986).

The injection of sedatives removes much of the variability associated with oral administration and results in shorter, more reliable induction periods. Injectable sedatives should be safe for human operators, have a wide safety margin, cause minimal pain on injection, have a small dose volume, have a rapid onset of action and result in a smooth induction and recovery (Vogelnest 1999). One commercial product that fits most of these criteria and is reported to be the sedative of choice for macropods is Zoletil® (Booth 1994; Vogelnest 1999). Zoletil® is a mixture of tiletamine, a

dissociative anaesthetic, and zolazepam, a derivative of benzodiazepine, which has been added to control the convulsions and irregular breathing that may be caused by tiletamine alone (Lin *et al* 1993). We have not been able to find reference to a universally reliable reversing agent for the mixture, and reversing either component alone risks unbalancing the mixture.

Widely differing dose rates have been reported for Zoletil® both between and within species (Lin *et al* 1993) and we have been unable to find documented dose-response data for western grey kangaroos. Booth (1994) recommended a dose of 10 mg kg^{-1} for marsupials in general, and while Vogelnest (1999) reported that an intramuscular dose of 5–15 mg kg^{-1} tranquillises macropods rapidly and smoothly, (Boever *et al* 1977) found that doses as low as 2–7 mg kg^{-1} sedated red kangaroos (*M. rufus*). Roberts *et al* (2010) used a dose of 125 mg for female and sub-adult male eastern grey kangaroos (*M. giganteus*), weighing approximately 13–36 kg, and a dose of 250 mg for larger males. The time for *Macropus* spp to recover completely from Zoletil® varies from half an hour to more than 24 h (Booth 1994), but it is not clear if that large variability is solely a dose-rate effect, as implied by Vogelnest (1999), or due to individual variability in susceptibility to Zoletil®, as implied by Booth (1994). We provide data on the variability in sedation time with Zoletil® dose in western grey kangaroos.

Materials and methods

The work in this study was carried out as part of a larger study and with the permission of the Department of Environment and Conservation (DEC), formerly the Department of Conservation and Land Management (CALM, Authority to enter CALM land and/or waters CE001513, CE001811, CE002253; Licence to take fauna for scientific purposes SF005629, SF006081, SF006652); and with the approval of the Animal Ethics Committee of The University of Western Australia (RA/3/100/592).

Study area

Harry Waring Marsupial Reserve (32°09'45"S; 115°49'51"E) covers an area of approximately 250 ha on the Swan Coastal Plain, 23 km south of Perth, Western Australia. A well-maintained, kangaroo-proof fence prevents public access and dispersal of resident western grey kangaroos but the kangaroos have free access to the entire reserve. As the kangaroos on the reserve had been habituated to twice-daily supplementary feeding throughout 2006, many could be readily approached to within 2 m while they were feeding and this facilitated injections into the large muscles of the hind limb using a 1.2-m pole-syringe.

Materials

Zoletil 100® (Virbac [Australia] Pty Limited, Milperra, Australia) is presented as 250 mg tiletamine and 250 mg zolazepam in a single vial. The contents of the vial are reconstituted with 5 ml of water for injection to produce 5 ml of 100 mg ml^{-1} active solution. The manufacturer recommends pre-medication with atropine to prevent salivation, but as this would be impractical with wild animals, we kept some atropine at-hand to treat excessive salivation if it became an issue.

Study animals

Over a period of 11 months, we sedated two young male western grey kangaroos and 24 adult females, up to four times each, at intervals of approximately four months, up to four at a time, using Zoletil® delivered by pole-syringe. We initially estimated that the weight of all the kangaroos we wanted to sedate was 20–30 kg, and used a dose of 1.25 ml (equal to 5 mg kg^{-1} for a 25-kg kangaroo) for each kangaroo. We subsequently varied this dose, but as our estimations of weight before injection were inaccurate, this did not reliably translate into lower dose rates. We laid each kangaroo on an electronic platform scale (150 × 90 cm; length × width) after it had been sedated, and recorded the live-weight to calculate the actual dose of Zoletil® used, and the time from injection to recumbency and recovery for each one. However, we were not able to weigh every kangaroo on the third occasion, and none on the fourth.

We laid kangaroos recovering from the Zoletil® in an open area and watched them continuously until they had recovered from the Zoletil®. We did not apply any tests or objective measurements during recovery to assess the kangaroos' level of consciousness, but assessed 'recovery' subjectively as the time that we felt that the animal was sufficiently co-ordinated to move without risk of injury due to drug-induced misadventure. Time to recovery therefore lacked the precision of an objective measurement and was rounded to the nearest quarter hour.

Because the kangaroos' eyes remain open whilst they are under the influence of Zoletil®, we placed a double layer of hessian under and over their head to protect and shade their eyes. In an effort to make the overall experience a positive one for the kangaroos and to encourage them not to try to hop away before they had completely recovered, we offered them a small amount of 'kangaroo muesli', a molasses-flavoured mixture of steam-rolled lupins, low-grade barley and sunflower seeds (Kangaroo Mix, Thompson & Redwood Produce Supplies, Upper Swan, WA, Australia), and lucerne hay as they regained consciousness. If any kangaroo tried to stand before it had regained adequate co-ordination, we steadied it by hand.

Statistical analysis

We used regression analysis to test for a relationship between dose rate and time to recovery for a total of 60 sedations and for the first sedation of all 26 kangaroos. We performed an analysis of variance with repeated measures followed by analysis for linear trend on the time to recovery (Schmuller 2009) for 17 kangaroos that were sedated three times to test whether they developed a tolerance for Zoletil®.

Results

With mean (± SD) Zoletil® dose rates of 4.55 (± 0.98) mg kg^{-1} by intramuscular injection on their first sedation, all kangaroos injected were quietly recumbent within 5–10 min, but some kangaroos were able to struggle inco-ordinately for up to 20 min if physically disturbed. Immediately after injection the injected kangaroo

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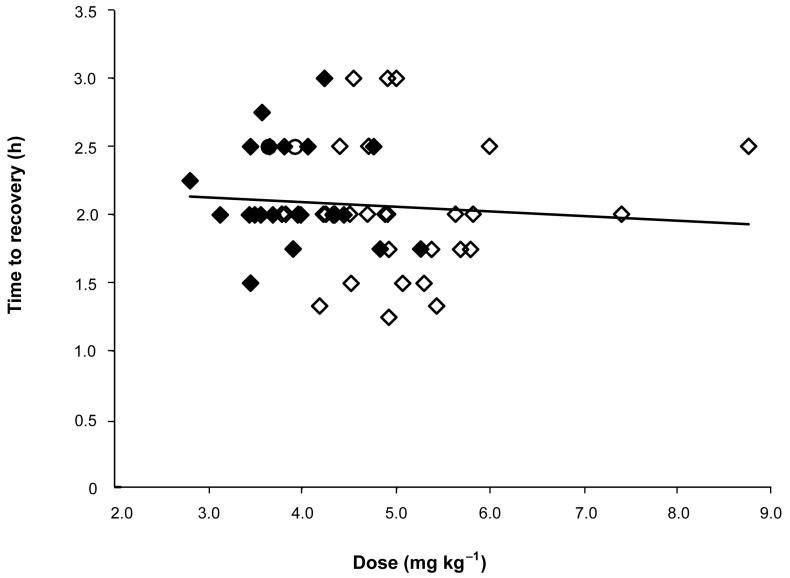
Figure 1

Time to recovery (h) for 24 adult female kangaroos sedated multiple times (first occasion [♦], subsequent occasions [◊]), and two young male kangaroos (one occasion each [○]), with varying dose rates (mg kg^{-1}) of Zoletil® by intramuscular injection (R^2 = 0.007, P = 0.86, df = 59).



and some of its close-by conspecifics usually hopped a short distance, up to about 10 m, but quickly returned to feeding, so it was possible to inject several kangaroos within about 10 min on each occasion.

As the Zoletil® took effect, most injected kangaroos fell quietly onto their side, but some dived forward and landed on one shoulder before kicking aimlessly a few times with their hind legs. They then lay quietly and would tolerate minor manipulation to varying degrees. For instance, opening of the pouch to check for presence of pouch-young, or collection of a blood sample from the jugular vein generally produced little or no response whereas puncturing of the ear to insert an ear tag in the same kangaroo, or attempts to collect blood from a lateral tail vein would invariably initiate two or three aimless kicks with the hind legs and raising of the head. We did not otherwise note any adverse effects, such as the hyper-responsiveness sometimes seen in horses and pigs when Zoletil® is used alone (Lin *et al* 1993), or excessive salivation. We therefore did not need to administer atropine to any of the kangaroos.

The mean (± SD) time to recovery, 2.07 (± 0.4) h, was independent of the dose rate (all sedations: n = 63, P = 0.39, R^2 = 0.01, df = 62; first sedation: n = 26, P = 0.55, R^2 = 0.02, df = 25 [Figure 1]; second sedation: n = 21, P = 0.1, R^2 = 0.14, df = 20; third sedation: n = 15, P = 0.09, R^2 = 0.20, df = 14) but mean (± SEM) time to recovery decreased with repeated uses of Zoletil (first occasion 2.25 [± 0.09] h; second occasion 2.15 [± 0.10] h; third occasion 1.81 [± 0.11] h; P = 0.003).

The behavioural characteristics of the recovery phase from sedation with Zoletil® were equally variable. Some kangaroos lay quietly and then rose gradually, from totally recumbent to up on one elbow, then to four feet and tail, before standing on two hind feet and tail and moving away quietly with the characteristic pentapedal steps of macropods moving slowly. These individuals all ate some of the kangaroo muesli, but none of the lucerne hay. Others rose quickly from totally recumbent to standing, and then tried to hop away. This usually resulted in a forward fall onto one shoulder and thrashing of the hind legs, or less often, a high jump with a backwards fall.

Almost all of the kangaroos we sedated were less wary of being approached after the event than before, making injection on subsequent occasions easier. However, one kangaroo became wary after two occasions and could not be approached to within 2 m after three occasions.

Discussion

We have shown that it is possible to selectively capture individual western grey kangaroos within a population by using intramuscular injections of Zoletil® at mean (± SD) dose rates of 4.55 (± 0.98) mg kg^{-1}, without inducing any overt signs of distress in either the captured kangaroos or their conspecifics. The level of sedation at this dose rate was adequate for minor manipulations such as morphometry and jugular venipuncture, while the period of sedation was free of undesirable side-effects such as hyperaesthesia or sialorrhoea. However, larger doses and/or the addition of a synergist such as xylazine or detomidine might be of benefit for conducting more invasive procedures. Although the time to recumbency was reliably 5–10 min, some kangaroos remained alert enough for up to 20 min to struggle incoordinately when physically disturbed. However, the time to recovery from the effects of Zoletil® was very variable, as has been found with eastern grey kangaroos (*Macropus giganteus*) (Roberts *et al* 2010) and the time to recovery was not related to dose rate. Based on these results there would appear to be little advantage in mixing Zoletil® with other drugs that may be reversed in an attempt to lower the dose rate of Zoletil® and thus shorten the time to recovery. Further, most other available drugs (or mixtures of

drugs) that can be at least partially reversed require larger dose volumes than Zoletil®, thus increasing the risk of incomplete injection and a period of excitement and flight before complete sedation is achieved.

The observed decrease in recovery times with repeated sedation with Zoletil® may have been due to physiological adaptation to the Zoletil® or the kangaroos may have learned and adjusted their behaviour to cope with Zoletil®-induced disorientation. Alternatively, it may reflect a change in how we dealt with them and our subjective assessment of their vulnerability. For instance, with experience, we may have acted sooner to steady those kangaroos that tried to stand too quickly, resulting in them being able to hop away at their first attempt rather than returning to recumbency. Although other researchers reported having given repeat doses of Zoletil® to some individual animals (Wan *et al* 1992; Massolo *et al* 2003), none suggested a physiological adaptation to Zoletil®. Future research using more objective measures of recovery might clarify the situation.

Animal welfare implications and conclusion

Western grey kangaroos can be reliably, quickly, safely and adequately sedated for minor procedures, such as ear-tagging, pouch inspection or collection of blood samples, with Zoletil® (tiletamine and zolazepam) at mean (± SD) dose rates of 4.55 (± 0.98) mg kg^{-1} by intramuscular injection. However, the time to recovery from Zoletil® can be prolonged and there is currently no effective reversing agent available for Zoletil®. As sedated kangaroos may be vulnerable to injury or predation due to disorientation or lack of co-ordination, we believe they should be supervised during recovery to prevent these possibilities and the recovery time should be factored into the planning of the procedures.

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