Tamoxifen exacerbates morbidity and mortality in mice receiving medetomidine anaesthesia

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Abstract
Tamoxifen-induced CreER-LoxP recombination is often used to induce spatiotemporally controlled gene deletion in genetically modified mice. Prior work has shown that tamoxifen and tamoxifen-induced CreER activation can have off-target effects that should be controlled. However, it has not yet been reported whether tamoxifen administration, independently of CreER expression, interacts adversely with commonly used anaesthetic drugs such as medetomidine or its enantiomer dexmedetomidine in mice. Here we report a high incidence of urinary plug formation linked to morbidity after ketamine-medetomidine anaesthesia in male mice previously injected with tamoxifen. As dexmedetomidine causes morbidity and mortality in male mice at higher rates than medetomidine, our findings suggest that both medetomidine and dexmedetomidine are both contra-indicated for male mice after tamoxifen treatment.

Introduction
The Cre-LoxP system is often used to induce gene deletion in mice, whereby the P1 bacteriophage Cre recombinase (hereafter referred to as Cre) is inserted together with a promoter into the mouse genome to excise the LoxP-flanked genetic material in cell types that express Cre. CreER is a modified version of Cre, in which Cre is fused to the oestrogen receptor (estrogen receptor, ER) to retain it in the cytoplasm, but CreER translocates to the nucleus after binding the tamoxifen metabolite 4-hydroxy tamoxifen (4-OHT). Thus, treating mice with tamoxifen or 4-OHT enables temporally controlled gene deletion.

Although CreER-mediated gene modification is now a widely used model to study gene function in the mouse, both tamoxifen and CreER induction have been reported to cause toxicity, with the extent of toxicity dependent on the tamoxifen dose, because tamoxifen itself can be toxic at high concentrations, but also because a higher tamoxifen dose induces more CreER nuclear translocation and thus more off-target effects. For example, the intraperitoneal injection of
pregnant dams with tamoxifen at embryonic day (E) 9.75 caused a high incidence of limb abnormalities in E17 mouse embryos. Tamoxifen also causes intrauterine haemorrhage and increases the mortality of pregnant mice injected at E5.5, but tamoxifen treatment of non-pregnant mice does not increase mortality.

CreER-modified transgenic mice may also be used in procedures that require anaesthesia after tamoxifen administration to induce gene deletion. Thus, the α2-adrenoreceptor antagonist medetomidine or its active enantiomer dexmedetomidine are commonly included in injectable anaesthetic regimes that are used in both veterinary medicine and research studies using live mice. However, it has not been reported whether tamoxifen exacerbates adverse effects of dexmedetomidine or medetomidine. Here we describe urinary plug formation after ketamine-medetomidine anaesthesia in tamoxifen-treated male mice. As dexmedetomidine can cause morbidity and mortality in male mice at higher rates than medetomidine, our findings suggest that medetomidine, even though preferable over dexmedetomidine in male mice, is also contraindicated for male mice after tamoxifen treatment.

Methods

Adult male and female mice (defined as over 2 months of age) on a mixed C57Bl/6J and 129/Sv background were housed in individually ventilated cages and fed with a regular chow diet. All animal work was carried out with a licence provided by the UK Home Office after local ethical and veterinary review. All mice described in this report were originally intended to provide controls for gene deletion experiments, or used to study the effects of gene deletion with Cre or CreER. 7 and 3 days, or 7 and 0 days, or 28 and 14 days prior to anaesthesia, mice were injected intraperitoneally on two occasions with 0.5 mg tamoxifen (Sigma-Aldrich) dissolved in 85 µl or 250 µl vegetable oil (Sigma-Aldrich). This tamoxifen dose was chosen as a reported effective dose to induce genetic deletion in adult mice with Cagg-CreER. As per local recommended anaesthetic practice to induce deep anaesthesia prior to surgery, mice were then given 75 mg/kg of ketamine (Narketan; Vetoquinol) and 0.5 mg/kg of medetomidine (Dormitor; Vetoquinol), both diluted in sterile water. Intraperitoneal injection of 8.3 mg/kg atipamezole (Antisedan; Zoetis) was used to resolve medetomidine action. Mice were kept on a warming pad until fully recovered from sedation. Mice were returned to their home cages and monitored daily for 7 days after anaesthesia. Mice were monitored daily for clinical signs such as hunching and grimacing, mobility and signs of a hard or distended bladder. Mice were euthanised by an appropriate schedule 1 method at the end of the experiment or upon reaching their humane endpoints. Humane endpoints were defined as an...
inability to urinate, severely reduced mobility or signs of distress or pain as evidenced by the grimace scale. Statistical analysis was performed using GraphPad Prism v9.2. Survival curves were analysed for difference using a Log-rank (Mantel-Cox) test. P-values of ≤0.05% were considered significant.

Results
Adult male and female mice were injected intraperitoneally with 0.5 mg tamoxifen on two occasions prior to anaesthesia with 75 mg/kg ketamine and 0.5 mg/kg medetomidine and the resolving anaesthesia using atipamezole (Fig. 1A). We unexpectedly observed that male mice injected with the second tamoxifen dose 0-3 days prior to induction of anaesthesia had a 50% incidence of adverse effects compared to 9.4% in mice that had received their second tamoxifen dose 14 days prior to anaesthesia, and male mice not given tamoxifen had no adverse effects (Fig. 1B, C). Severe adverse effects were seen in male mice expressing CreER and in CreER-negative control male mice. Moreover, female mice showed no obvious adverse effects, regardless of their tamoxifen dosing schedule (Fig. 1B, C). Mice found at their humane endpoints presented with an inability to urinate when scruffed. Of the male mice euthanised at their humane endpoint, 3 mice were analysed post-mortem for gross abnormalities, and all were found to have distended bladders (Fig. 1D). Some mice were found dead, thereby exceeding the pre-specified humane endpoint of the study, requiring the filing of a report sent to the named animal welfare office, local veterinarian and legislator. Specifically, we observed a significantly decreased survival rate up to 72 hours post-anaesthesia in male mice injected with tamoxifen compared to male mice not injected with tamoxifen (Fig. 1B). By contrast, female mice did not have decreased survival after tamoxifen dosing followed by anaesthesia (Fig. 1B).

Note: This report was produced to document and alert other researchers to the adverse effect of tamoxifen administration prior to medetomidine anaesthesia in male mice. We will not repeat the above experiments to increase statistical power for ethical reasons.

Discussion
Our study has established that the combination of tamoxifen injection with the anaesthetic agent medetomidine increased mortality in male mice 24-72 hours post-anaesthesia. Medetomidine is comprised of a combination of 2 enantiomers, dexmedetomidine and levomedetomidine, whereby dexmedetomidine is thought to be the active form and typically used at half the dose of medetomidine 10. Both medetomidine and dexmedetomidine have been suggested to be
comparable in anaesthetic effect, and each needs to be combined with ketamine for deep anaesthesia. Even though medetomidine and dexmedetomidine are commonly used in both veterinary medicine and research with live mice, a literature search after observing adverse effects identified two published studies reporting that medetomidine and dexmedetomidine cause mortality in C57Bl/6J male mice at an incidence of 3% and 67%, respectively. In agreement with the lower incidence of 3% mortality with medetomidine compared to dexmedetomidine, we did not observe any deaths in the small cohort of male mice that had received ketamine-medetomidine but no other intervention. By contrast, we observed increased mortality in 50% of male mice treated with tamoxifen within 3 days and in 9.4% of male mice treated with tamoxifen within 14 days of receiving medetomidine. These findings suggest that tamoxifen exacerbates medetomidine toxicity and that a shorter interval between administering both drugs is the most detrimental.

Medetomidine has been used in a range of domestic animal species for veterinary semen collection with a catheter under anaesthesia and has been associated with increased sperm counts. In agreement, the prior study reporting increased mortality in male mice linked this to the formation of seminal coagulum that blocked the urethra, presumably because ejaculation was stimulated but the ejaculate was not released or manually removed, as is the case in veterinary practice for semen collection. As previously described for medetomidine-anaesthetised male mice in a study not using tamoxifen, the medetomidine-anaesthetised male mice treated with tamoxifen in our study also had distended bladders on post-mortem. Nevertheless, it is unclear why tamoxifen treatment increased the incidence of this medetomidine-induced adverse effect. As tamoxifen treatment in male mice decreases the proportion of mature elongated sperm cells at the expense of rounded, immature spermatogonia in the Sertoli cells, it is conceivable that immature sperm cells released into the seminal fluid may increase the incidence of urethral plug formation.

It cannot formally be excluded that elements of the anaesthetic regimen other than medetomidine increased mortality in tamoxifen-treated mice, or that mechanisms other than seminal coagulum are responsible; however, both possibilities seem unlikely, because female mice did not show mortality in our study. Moreover, a prior report showed that medetomidine toxicity in male mice was mitigated by replacing medetomidine with xylazine, whilst retaining ketamine and atipamezole in the anaesthetic protocol. Further work would be required to determine whether tamoxifen can also exacerbate the side effects of other anaesthetic regimens.

Notably, the mortality and morbidity associated with tamoxifen use prior to medetomidine-based anaesthesia decreased with a longer duration between the last tamoxifen dose and the induction of anaesthesia. Thus, mortality in male mice was reduced from 50% to 9.4% when the last
dose of tamoxifen was administered 14 days before anaesthesia, as opposed to 0 or 3 days before anaesthesia. Nevertheless, we consider a 10% mortality incidence unacceptable. An even longer duration between tamoxifen administration and induction of anaesthesia may further reduce the mortality rate but may be unsuitable for some experimental models due to recovery from the intended purpose of tamoxifen administration, which is to induce gene deletion. Thus, a population of recombination-resistant cells may have an advantage over recombined cells and out-compete them (e.g. Fantin et al. Blood, 2013). Therefore, we suggest that alternative injectable anaesthetic formulations not containing medetomidine should be used when using male mice, particularly in combination with tamoxifen administration to induce gene deletion, and when inhalation anaesthesia is unsuitable.

Declaration of conflicting interests: The authors declare that there is no conflict of interest.

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References:

10.1161/CIRCRESAHA.120.317025.


Figure 1: Tamoxifen injection followed by dexmedetomidine-ketamine anaesthesia causes morbidity and mortality in adult male but not female mice.

Mice were either not injected (n = 18 males, n = 6 females) or injected intraperitoneally with tamoxifen (n = 12 males, n = 15 females) prior to anaesthesia with ketamine-dexmedetomidine (n = 32 males, n = 17 females). Mice were monitored daily for signs of adverse effects.

(A) Treatment and analysis timeline: In some studies, mice were injected with tamoxifen on day 0 or 3, and on day 7 prior to anaesthesia. In other studies, mice were injected intraperitoneally with tamoxifen on days 14 and 28 prior to anaesthesia with ketamine-dexmedetomidine. Where mice were injected with tamoxifen on day 0, tamoxifen was given prior to anaesthesia. Male mice injected with tamoxifen on days 0/3 and 7 prior to anaesthesia had significantly reduced survival compared to un.injected male mice (6 out 12 mice lost, P=0.0009). Male mice injected with tamoxifen on days 14 and 28 prior to anaesthesia did not have significantly different survival compared to un injected male mice (3 out of 32 mice lost, P=0.1861).

(B) Survival curves of male and female mice up to 7 days after anaesthesia.

(C) Proportion of adverse effects in uninjected and tamoxifen-injected male and female mice within 7 days of anaesthesia.

(D) Example of a male mouse culled due to severe adverse effects, sprayed with ethanol before dissection to prevent fur shedding; the arrowhead indicates a distended bladder.