Title

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Journal

Epilepsia open, 8(2)

ISSN

2470-9239

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Publication Date

2023-06-01

DOI

10.1002/epi4.12723

Peer reviewed

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Tolerability and pharmacokinetics of intravenous allopregnanolone with and without midazolam pretreatment in two healthy dogs

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/epi4.12723

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Key words: seizure, status epilepticus, allopregnanolone, midazolam, cardiopulmonary

Number of text pages: 8

Number of text words: 1843

Number of references: 23

Number of figures: 3

Number of tables: 0

Number of supporting informational figures: 1

Number of supporting informational tables: 2

SUMMARY

The neurosteroid allopregnanolone (ALLO) is under investigation as a treatment for benzodiazepine-refractory status epilepticus (SE). Here we assess the cardiopulmonary safety of intravenous ALLO by itself and after a clinically recommended dose of midazolam (MDZ) in two healthy adult beagles. Each dog received ALLO (1 mg/kg, IV), and after a washout period of 2 weeks, the dog was dosed with MDZ (0.2 mg/kg, IV) followed 10 min later by ALLO. Behavioral state, vital signs, arterial blood gasses, blood chemistries, and plasma ALLO concentrations were monitored for up to 6 h after dosing. The dogs appeared sleepy but were fully responsive after both treatments. No depression of mean arterial pressure or respiratory rate was noted. Blood gas measurements failed to show evidence of drug-induced acute respiratory acidosis. Estimated maximum plasma ALLO concentrations were in the range of 1500 to 3000 ng/mL. The results indicate that intravenous ALLO can be used safely to treat benzodiazepine-refractory SE, even when administered shortly after a benzodiazepine.

Key words: seizure, status epilepticus, allopregnanolone, midazolam, cardiopulmonary

INTRODUCTION

Benzodiazepines, including the rapidly acting water-soluble benzodiazepine midazolam (MDZ)[1, 2], are the recommended first line treatment for status epilepticus (SE) in both human and veterinary medicine[3]. Benzodiazepines become less effective with increasing time after SE onset[4] and often fail to terminate SE[5]. When one or more benzodiazepine doses do not stop seizures, other antiseizure medications are administered intravenously, and if these also fail, general anesthesia may be required[6, 7]. Continuing seizure activity and the effects of the various treatments are associated with increased risk of poor outcome. New therapeutic agents or combinations that effectively terminate benzodiazepine-refractory SE and avoid the adverse physiological effects of general anesthetics are urgently needed to improve long-term outcomes.

Allopregnanolone (ALLO) is an endogenous neurosteroid that acts as a positive allosteric modulator of $GABA_A$ receptors. ALLO has several pharmacological properties that are distinct from benzodiazepines, which also positively modulate $GABA_A$ receptors[8]. ALLO has powerful antiseizure activity and in experimental animal models of chemically-induced SE has been shown to significantly delay seizure onset, reduce seizure incidence and duration, terminate electroencephalographic epileptic discharges, and protect against neuronal calcium dysregulation, particularly when used in combination with or following a benzodiazepine[9-12]. Importantly, ALLO has been found to effectively terminate SE in animals that fail to respond to MDZ at doses that are associated with plasma levels comparable to or substantially greater than those achieved in humans administered MDZ at doses recommended for the treatment of

SE[11]. These observations suggest that ALLO could be used to terminate SE in benzodiazepine-refractory patients.

Recognizing that both MDZ and ALLO can potentially depress respiration and blood pressure[13, 14], we sought to assess the cardiopulmonary safety of a staged treatment approach whereby the first drug administered is standard-of-care MDZ followed by ALLO as a second agent. The study was carried out in two healthy dogs. We used MDZ at the recommended intravenous bolus dose for the treatment of SE in dogs (0.2 mg/kg)[15] and an intravenous dose of ALLO (1 mg/kg) predicted to produce plasma levels in dogs associated with therapeutic activity in animal models of benzodiazepine-refractory SE. In clinical practice, a second treatment agent is administered as soon as possible after treatment failure is recognized. Considering that SE is commonly defined as seizure activity of 5 min or more and assuming a lag of 5 min to administer the second treatment, we imposed an interval of 10 min between the initial MDZ dose and the ALLO dose[16].

METHODS

Two beagles (Marshall BioResources), a 1-year-old, 9.2 kg unneutered male and a 5-year-old, 12.2 kg unneutered female, were housed individually at an AAALAC International-accredited facility at the University of California, Davis (UC Davis). Dogs were handled for at least 1 h per day for 2 weeks prior to the study to reduce stress. The study adhered to the ARRIVE guidelines and the NIH Guide for the Care and Use of Laboratory Animals (NIH publication No. 8023, revised 1978) and was approved by the UC Davis IACUC (protocol # 21966).

Dogs first received ALLO (1 mg/kg, IV) alone, and then after a 2 week drug washout period, MDZ (0.2 mg/mL, IV; West-Ward) followed 10 min later by ALLO (1 mg/kg, IV). ALLO (99.9% pure) was dissolved in 0.9% NaCl in 40% hydroxypropyl-β-cyclodextrin (Captisol; CyDex). Drugs were administered via an IV catheter placed in the cephalic vein 1 h before the first drug dose and were injected by rapid IV push over a period of <30 sec. A second catheter was placed in the metacarpal artery in the contralateral limb for blood collection. A baseline blood sample was collected prior to dosing.

Vital sign data, including heart rate, respiratory rate and mean arterial pressure were recorded at baseline prior to dosing (designated time 0) and 2, 5, 10, 15, 20, 30, 45 min and then hourly from 1-6 h post-treatment. Heart rate and blood pressure were evaluated using a Berry Electronic Technology pressure cuff. Five individual readings were collected at each time point, and the data were averaged. The Modified Glasgow Coma Score was used to monitor the level of consciousness.

Approximately 2 mL of blood was drawn from the indwelling arterial catheter at 0.5, 1, 2, 5, 10, 15, 20, 30 and 45 min and hourly from 1-6 h post-treatment for blood chemistry, hematology, and ALLO analysis. Whole arterial blood samples were analyzed immediately after collection using an i-STAT handheld critical blood analyzer (Abbott). Calibration standards were run before each study. Electrolytes (sodium, potassium), glucose, hematology (hematocrit, hemoglobin), and blood gases (pH; partial pressure of carbon dioxide, $PaCO_2$; partial pressure of oxygen, PaO_2; calculated bicarbonate, HCO_3; calculated total CO_2, TCO_2; calculated base excess; and calculated oxygen saturation, SaO_2) were recorded in each sample.

Plasma samples collected following whole blood centrifugation were frozen at –80 °C until analyzed. As described previously, an ultra-performance liquid chromatography-mass spectrometry method developed and validated at the UC Davis Bioanalysis and Pharmacokinetics Core Facility was used to measure total plasma ALLO concentrations[17]. Normal beagle plasma served as the control.

RESULTS

While both dogs exhibited sleepiness following administration of ALLO alone or with MDZ, they remained responsive. The Modified Glasgow Coma scores for motor activity and brain stem reflexes, which range from 6 for normal to 1 for comatose, were all ≥ 5 for the first hour (depressed consciousness and slow pupillary light reflex noted at times) and then 6 thereafter.

For the majority of the 6 h study, vital signs hovered near or stayed within normal reference ranges[18] with both treatments (Figure S1). Dogs exhibited a slight tachycardia immediately following dosing, perhaps because of behavioral stimulation or stress, but heart rate returned to normal within 30 min. Mean arterial pressure fluctuated around the reference range of 80-125 mm Hg[19] with both treatments. Respiratory rate remained largely within the reference range of 18-34 breaths/min[20] for both dogs, with a tendency for higher rates in the first hour after dosing and lower rates during the remaining period when the animals were less stimulated.

HCO_3 levels were in the reference range (18.3-26.4 mmol/L[21]) in both dogs regardless of treatment (Figure 1A,B). $PaCO_2$ levels were within the reference range (27.8-47.2 mm Hg[21]) for the female following either treatment (Figure 1C,D). In contrast,

the male had elevated $PaCO_2$ levels following administration of ALLO only (Figure 1C) for almost the entire experiment. However, the baseline $PaCO_2$ level was also elevated, so the high values could not be attributed to the ALLO treatment. Following combined treatment with MDZ and ALLO, the male's $PaCO_2$ levels remained within the reference range (Figure 1D). Both dogs had increased PaO_2 (reference range 85-100 mm Hg[21]) following ALLO alone and sequential MDZ and ALLO (Figure 2A,B) at most time points. SaO_2 levels remained well above 95% in both dogs following both treatments, except for a single measurement in the female dog of 94% (Figure 2C,D).

Blood electrolyte (sodium and potassium), chemistry (glucose, pH, TCO_2, base excess) and hematology (hematocrit and hemoglobin) values were recorded at each time point and compared to normal canine reference ranges[21] (Table S1). The male's sodium, potassium, and hematocrit lower bound concentration values fell just outside the corresponding reference ranges following treatment with ALLO only. The pH measurements in the male were in the acidotic range at some time points whereas this was not the case for the female. However, in both animals PCO_2 and HCO_3 were not elevated, indicating that respiratory acidosis was not present. Following MDZ and ALLO treatment, some potassium measurements in both animals were slightly below the reference range as was pH and hematocrit in the male.

Plasma ALLO concentrations are plotted in Figure 3A,B. Noncompartmental PK parameters derived from these plots are given in the table below. Exposure to ALLO as determined by the area under the curve (AUC) was greater in the female than in the male, which may reflect reduced clearance in the female (Table S2). For both dogs, ALLO exposure was greater when ALLO was administered following MDZ. Based on

the $AUC_{t-\infty}$ values, the difference was 44% in the male dog and 13% in the female dog. ALLO volume of distribution and clearance (CL) parameters estimated by two-compartment modeling are shown in Table S2.

DISCUSSION

Intravenous ALLO is a potential treatment for benzodiazepine-refractory SE that would be administered after the failure of a benzodiazepine, such as MDZ. Parenteral MDZ has been associated with respiratory depression[13, 14]. There is concern that concomitant use of ALLO, which is a central nervous system depressant, would increase the risk of cardiopulmonary adverse events, particularly at the high doses required to terminate SE. Therefore, we evaluated the cardiopulmonary safety of intravenous ALLO delivered alone or shortly after intravenous MDZ in two dogs. The dose of ALLO (1 mg/kg) was selected based on a two-compartment model using results from a prior study in dogs[22]. We intended to target a C_{max} value of approximately 2000 ng/mL because peak plasma concentrations of ALLO of approximately 600-2000 ng/mL can effectively terminate benzodiazepine-refractory SE in rodent models[17, 23]. We obtained estimated C_{max} values in the range of 1500-3000 ng/mL, validating our modeling. The C_{max} value predicted by simulation in the earlier study[22] for a 1 mg/kg dose (~1000 ng/ml) is below the range obtained in the present study, likely because the dose was infused over 5 min rather than as a rapid bolus injection as was done here.

The two dogs used in this study, a 1-year-old male beagle and a 5-year-old female beagle, did not suffer adverse vital signs (heart rate, mean arterial pressure, respiratory rate) or blood gas levels (PaO_2 and SaO_2). For the male dog following ALLO

only, $PaCO_2$ values were consistently in the hypercapnic range (>45 mmHg) but there was no increase in HCO_3 as would occur in acute respiratory acidosis. Moreover, the $PaCO_2$ level was elevated at baseline, suggesting that the elevated values were not related to the treatment. Importantly, $PaCO_2$ was not abnormal in the same dog when ALLO was administered after MDZ treatment.

For both dogs, exposure to ALLO was greater when ALLO was administered following MDZ. MDZ is well recognized as a CYP3A4 substrate, and in vitro studies demonstrate that ALLO is strongly metabolized by CYP3A4 (FDA Drug Approval Package for Zulresso). However, MDZ is not known to affect the pharmacokinetics of other drugs, so an interaction between MDZ and ALLO requires further study. ALLO is not known to be a CYP3A4 inhibitor, so its action as a perpetrator on MDZ metabolism is not expected.

A major limitation of this study is the small number of animals used; thus, we did not attempt to combine the PK results, but rather reported them separately for each dog. Confirmation of these preliminary results is required. We also did not have a vehicle control, so we do not know to what extent the stress of the IV injections contributed to changes in vital signs or blood gas values.

CLINICAL RELEVANCE

ALLO may be an effective treatment for benzodiazepine-refractory SE. In this situation, patients will have received a benzodiazepine as initial treatment. The present results indicate that intravenous ALLO is likely to be safe even when administered shortly after the benzodiazepine.

Acknowledgments: This work was funded by UC Davis School of Veterinary Medicine Center for Companion Animal Health (CCAH grant # 2020-20-F). We thank the UC Davis Campus Veterinary Services for their assistance in socializing and caring for the animals as well as adopting them out at the end of the study.

Conflicts of Interests: Michael A. Rogawski, Dorota Zolkowska and Pamela J. Lein are named inventors of patents and patent applications assigned to the Regents of the University of California claiming the use of neurosteroids to treat status epilepticus. The other authors declare that they have no conflicts of interest.

Ethics Publication Statement: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Author Contributions: DAB acquired and analyzed blood gas and physiologic data and drafted the manuscript; BM and YJC trained the dogs and acquired the clinical and pharmacological data; CYW performed dose selection and conducted bioanalysis, pharmacokinetic modeling and analysis; MA assisted with clinical interpretation and revising the manuscript; DZ prepared the treatment solutions and was involved in the design of the study; MAR was involved in conception and design of the study and drafting the manuscript; SSJ helped draft the manuscript; PJL participated in the

conception and design of the study and drafting the manuscript. All authors read and approved the final manuscript.

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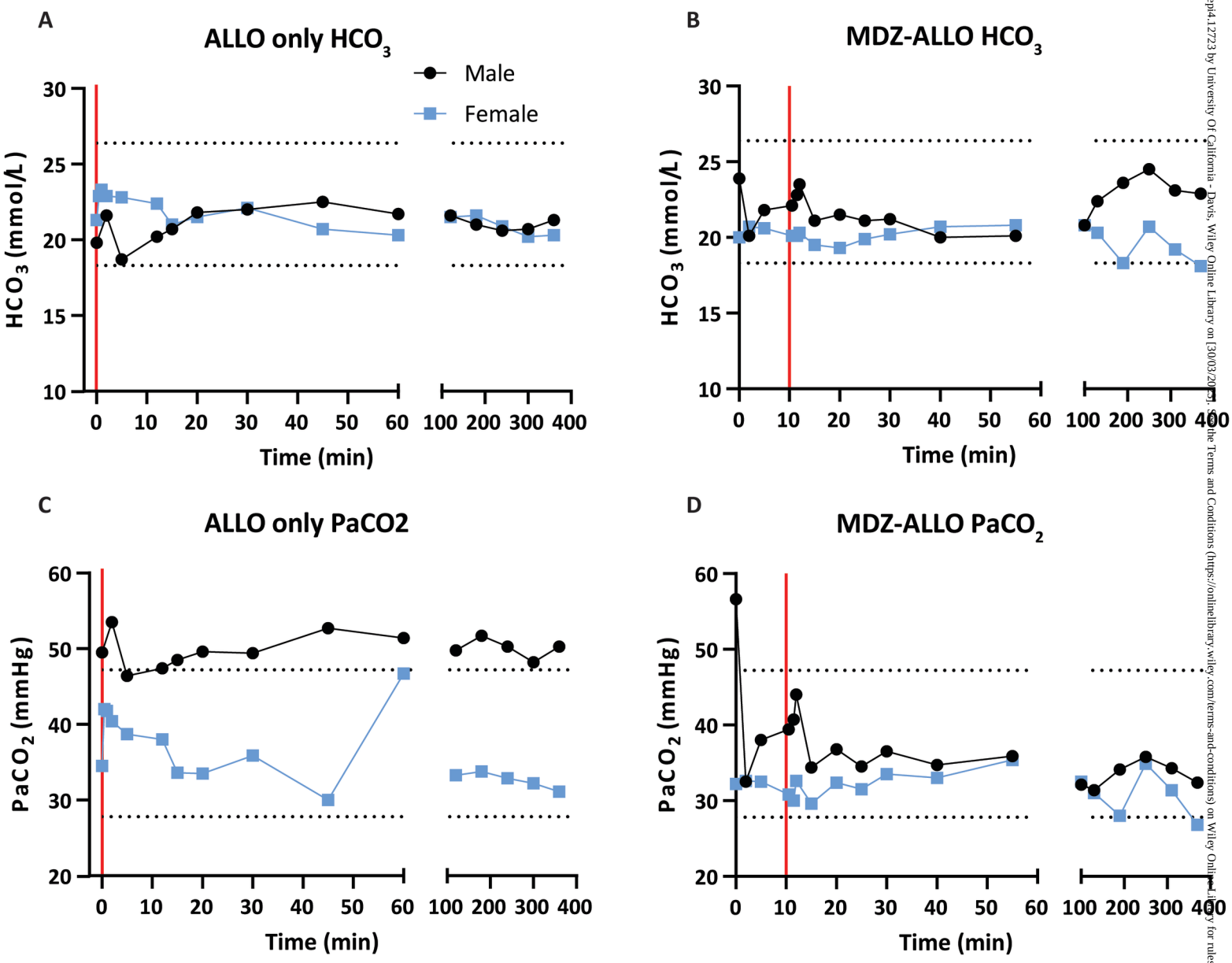
FIGURE LEGENDS

Figure 1. Calculated arterial blood bicarbonate (HCO_3) and partial pressure of carbon dioxide ($PaCO_2$) immediately before (time 0) and at intervals up to 6 hours. In the experiments of A and C, allopregnanolone (ALLO; 1 m/kg, IV) was administered at time 0. In B and D, midazolam (MDZ; 0.2 mg/kg, IV) was administered at time 0 followed by ALLO at 10 min. Red vertical lines indicate ALLO administration. Dotted horizontal lines indicate upper and lower values of the reference range.

Figure 2. Calculated arterial blood partial pressure of oxygen (PaO_2) and saturated oxygen (SaO_2) immediately before (time 0) and at intervals up to 6 hours. In the experiments of A and C, allopregnanolone (ALLO; 1 m/kg, IV) was administered at time 0. In B and D, midazolam (MDZ; 0.2 mg/kg, IV) was administered at time 0 followed by ALLO at 10 min. Red vertical lines indicate ALLO administration. Dotted horizontal lines indicate upper and lower values of the reference range.

Figure 3. Plasma allopregnanolone (ALLO) concentration-time profiles following ALLO (1 mg/kg, IV) alone (A) or when administered 10 min after midazolam (MDZ; 0.2 mg/kg, IV) (B). Noncompartmental pharmacokinetic parameter estimates are shown in the table. C_{max}, estimated maximum plasma concentration determined by extrapolation to time 0 using a two-compartment model simulation with parameters as shown in Table S2; T_{last}, time of last measurable concentration; AUC_{last}, area under the concentration-time curve (AUC) from dosing (time 0) to the time of the last measured concentration;

$AUC_{t-\infty}$; AUC from dosing (time 0) extrapolated to infinity; Fraction AUC extrapolated, fraction of total AUC due to the extrapolated AUC.



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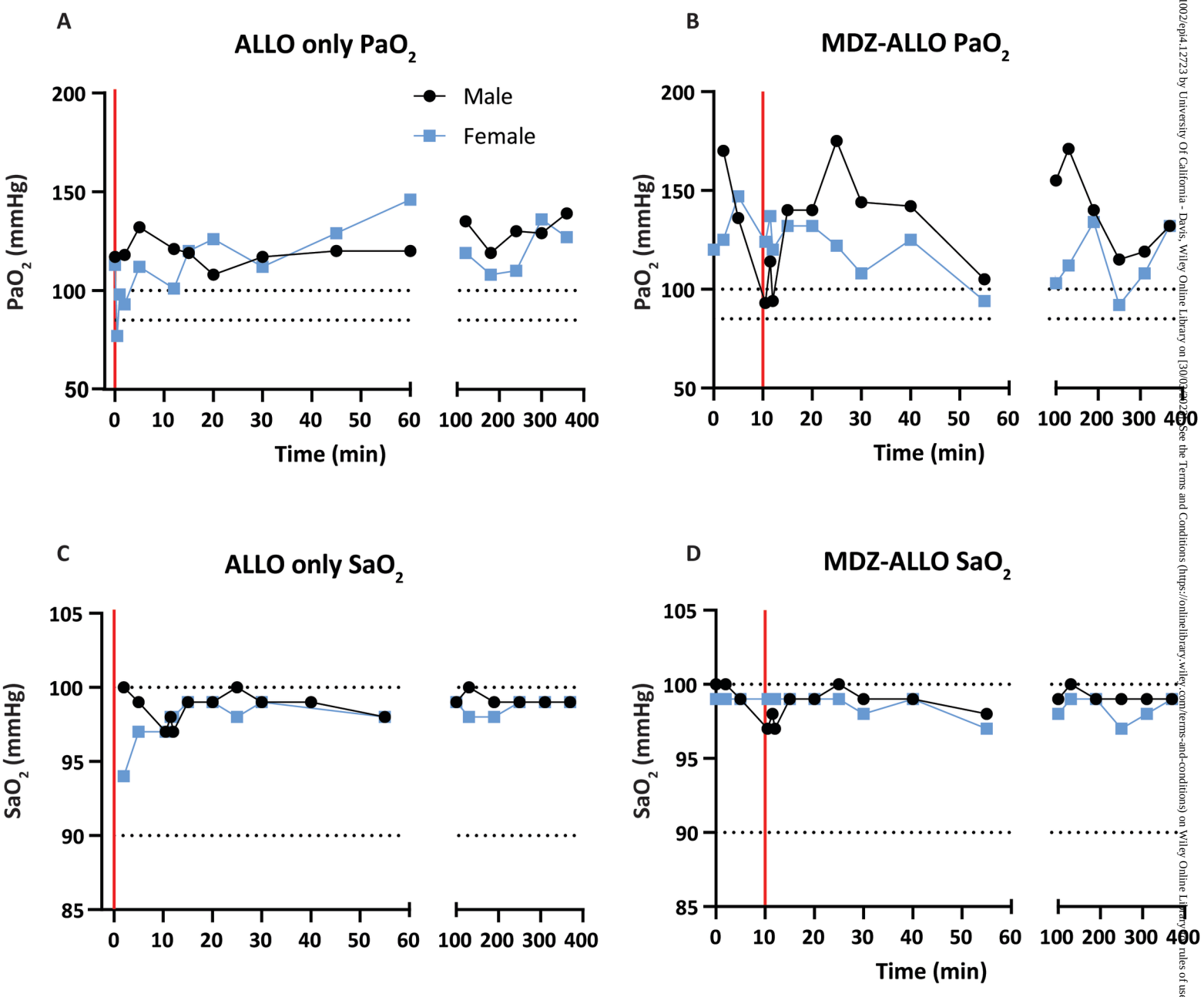

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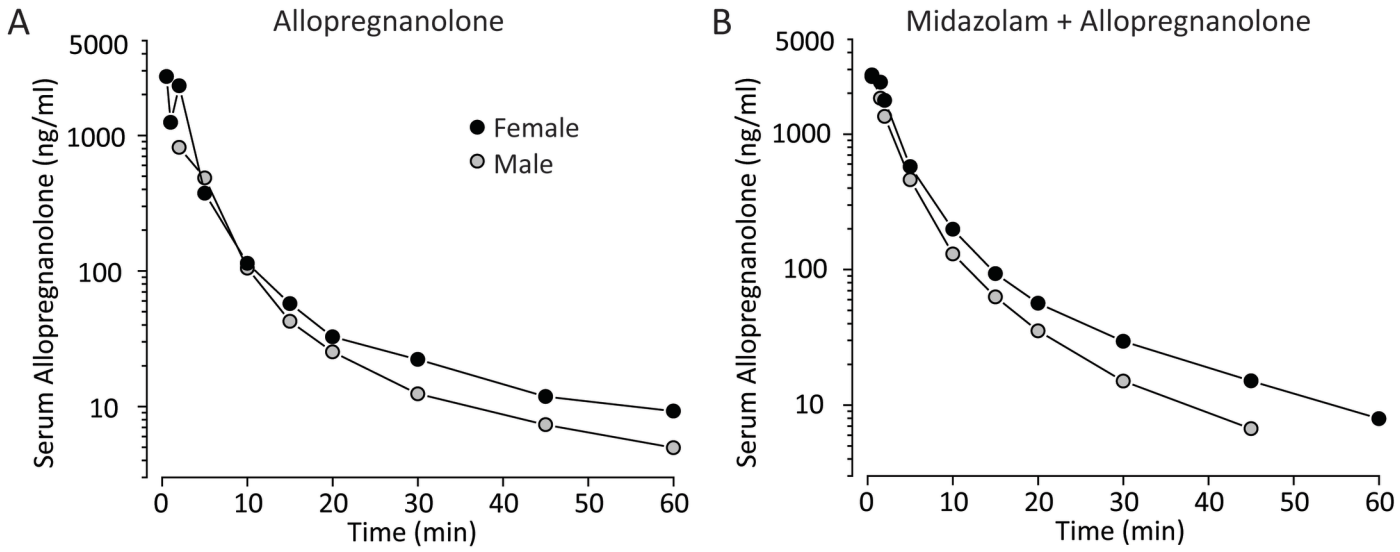

Subject	Treatment	C_{max} (ng/ml)	T_{max} (ng/ml)	$t_{1/2}$ (min)	T_{last} (min)	AUC_{last} (min ng/ml)	$AUC_{t-\infty}$ (min ng/ml)	Fraction AUC extrapolated (%)
Male	ALLO	1570.3	0.0	22.7	60.0	6028.7	6190.8	2.6
	MDZ + ALLO	3061.2	0.0	10.6	45.0	8821.9	8924.2	1.2
Female	ALLO	3144.2	0.0	35.3	120.0	10713.9	10851.2	1.3
	MDZ + ALLO	3150.9	0.0	21.1	90.0	12112.7	12236.0	1

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