

CASE REPORT

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Unexpected effects of ivermectin and selamectin on inducible Cre^{ER} activity in mice

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Abstract

Background: Anti-parasitics are frequently used in research animal facilities to treat a multitude of common infections, with pinworms and fur mites being amongst the most common. Ivermectin and selamectin are common oral and topical treatments for these infections, respectively. Although commonly thought to be innocuous to both the research animals and any transgenic elements that the animals may carry, evidence exists that ivermectin is capable of activating the recombinase activity of at least one Cre^{ER}. The goal of the current study was to determine if there was an effect of either anti-parasitic agent on the activity of Cre^{ER} proteins in transgenic mice.

Case presentation: We analyzed the offspring of transgenic mice exposed to either ivermectin or selamectin during pregnancy and nursing. Through analysis of reporter genes co-expressed with multiple, independently generated transgenic Cre^{ER} drivers, we report here that ivermectin and selamectin both alter recombinase activity and thus may have unintended consequences on gene inactivation studies in mice.

Conclusions: Although the mechanisms by which ivermectin and selamectin affect Cre^{ER} activity in the offspring of treated dams remain unclear, the implications are important nonetheless. Treatment of pregnant transgenic mice with these anti-parasitics has the potential to alter transgene activity in the offspring. Special considerations should be made when planning treatment of transgenic mice with either of these pharmacologics.

Keywords: Ivermectin, Selamectin, Transgenic mice, Cre^{ER}

Background

Helminth infection is one of the most common types of parasitic infection in laboratory rodents. Although normally benign, these infections can eventually cause secondary effects including, but not limited to, heightening the host animal's immune system. Such effects can not

only confound studies of the immune system, but also alter the activity of any exogenous treatment or genetic element potentially recognized as foreign. Given these complications, in addition to the obvious problem of parasite burden, treatment of laboratory rodent colonies for removal of parasites is imperative for institutional veterinary care and research alike.

Amongst the most common treatments for helminthic parasitic infections are the pharmacological agents ivermectin and selamectin. Both drugs treat existing helminth infections by inducing paralysis in the parasites and thereby preventing normal life functions. Ivermectin

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is ingested orally whereas selamectin is administered topically. The commonly held belief is that ivermectin has minimal effect on mammalian systems and can thus be used without ill-effect on rodent colonies during ongoing pre-clinical studies. While this understanding of the safety of ivermectin appears to hold true in most circumstances, there is evidence that ivermectin treatment can have unintended effects on certain transgenic elements [4]. Variants of Cre recombinase, an enzyme used to conditionally inactivate genes, that are fused to a mutated form of the estrogen receptor (ER) and allow for temporal control of gene inactivation seem to be most sensitive to these treatments. Known as a Cre^{ER}, this fusion protein is activated upon the administration of tamoxifen, an estrogen analog, resulting in translocation of the Cre^{ER} protein to the nucleus where it mediates DNA recombination at specific sequences known as *loxP* sites introduced into the desired locus.

A previous study has shown that ivermectin treatment in mice is capable of constitutively activating a Cre^{ER} specifically expressed in T-lymphocytes [4]. Given the concern this raises for temporal and cell type-specific regulation of gene inactivation, we performed independent pilot studies to examine the potential effects of ivermectin on other Cre^{ER} transgenes prior to a facility-wide treatment at our institution. Here we report the paradoxical findings that ivermectin and selamectin treatments could either aberrantly activate or prevent the activity of three independently generated transgenic Cre^{ER} fusion proteins.

Case presentation

First, we examined the activity of an unpublished transgenic Elastase1-Cre^{ER} (Ela1-Cre^{ER}) expressed in pancreatic acinar cells where the *elastase1* promoter drives expression of the Cre^{ER} (gift from Dr. Steve Konieczny, Purdue University). This transgene is expressed at such a high level, due to the strength of the promoter, that it has constitutive activity in approximately 50% of pancreatic acinar cells as evidenced by activation of a *lox-stop-lox-yellow fluorescent protein* (*lsl-YFP*) reporter construct knocked into the ubiquitously-expressed *Rosa26* locus [9]. In this construct, the *stop* sequence prevents transcription of the YFP mRNA. Activity of Cre recombinase removes the *stop* sequence thereby allowing transcription and translation of YFP and detection by live imaging or immunofluorescence. As noted above, the constitutive activity of the Ela1-Cre^{ER} normally results in YFP protein expression and detection in ~50% of pancreatic acinar cells even in the absence of tamoxifen.

For the purposes of this experiment, female mice homozygous for the *lsl-YFP* were mated to male mice carrying the Ela1-Cre^{ER}. Following mating, female mice were placed on either standard chow diet (Lab Diet

5L0D, PMI Nutrition) or the diet containing ivermectin (Lab Diet 5L0D 12 ppm ivermectin, PMI Nutrition). Parental mice were maintained on the appropriate diet throughout pregnancy, birth of the pups, and until weaning at 21 days. At weaning, the pups were sacrificed, pancreata were harvested, and tissue sections were examined via immunofluorescent labeling for YFP (as in [6]). Unfortunately, one of the dams carrying the Ela1-Cre^{ER} transgene ate her pups, so we were limited to examining one litter of mice exposed to ivermectin. In this litter, 3/9 mice did not carry the Ela1-Cre^{ER} transgene and 6/9 mice carried both the Ela1-Cre^{ER} transgene and the *lsl-YFP* reporter (double transgenic). Normally, all six of these mice were expected to have detectable YFP expression. Indeed, all mice with both the transgene and reporter that were raised on the standard chow diet had detectable reporter expression (7/7 analyzed). Of the six double transgenic mice raised on ivermectin diet, 2/6 maintained YFP expression. However, 4/6 double-transgenic mice raised on the ivermectin diet had no detectable YFP expression (Fig. 1) indicating that, by an unknown mechanism, expression, or activity, of the Ela1-Cre^{ER} is reduced or absent. The discrepancy between these findings and the findings of Corbo-Rodgers et al cannot be reconciled at this point since no mechanism of interaction between ivermectin and Cre^{ER} proteins has been identified.

The second experiment analyzed the activity of two Cre^{ER} transgenes expressed in the brain that label distinct neural stem cell populations within the ventricular-subventricular zone (V-SVZ). Empty spiracles homeobox 1 (Emx1-Cre^{ER}) [5] is expressed in the developing cerebral cortex and in stem cells of the dorsal V-SVZ while GLI family zinc finger 1 (Gli1-Cre^{ER}) [1] is expressed predominantly in the ventral forebrain, including the ventral V-SVZ. This experiment utilized a similar reporter to the one detailed above, but the fluorophore was a tdTomato following the *lsl* sequence in the *Rosa26* locus. This specific reporter is known as Ai14 [7]. Gli1-Cre^{ER}; Ai14 and Emx1-Cre^{ER}; Ai14 lines were either untreated, received an ivermectin diet (as above), or a topical selamectin treatment (Revolution, 0.3 mg, Zoetis, Inc) with fenbendazole diet (Lab Diet 5L0D, 150 ppm fenbendazole). Our normal experience with these lines is that no reporter gene expression is observed in the absence of tamoxifen administration. Ivermectin or selamectin treatment began at the start of gestation and continued in the postnatal period until animals were sacrificed at postnatal day 7 at which time brain slices were prepared for imaging. For all breeding pairs, Cre^{ER}-negative females were paired with Cre^{ER}-positive males. At no point were animals exposed to tamoxifen.

From the Emx1-Cre^{ER}; Ai14 line, we obtained two pups carrying both the Cre^{ER} and the *lsl-tdTomato*

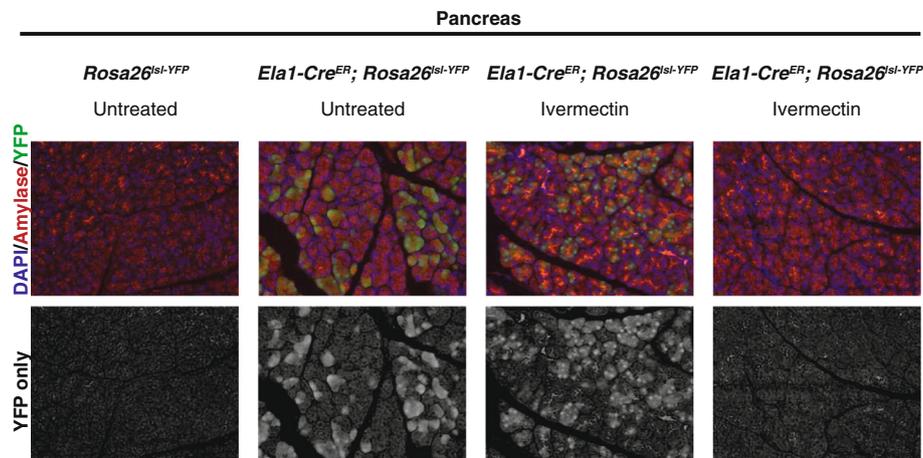


Fig. 1 Ivermectin-induced silencing of *Ela1-Cre^{ER}* in the pancreas. Representative immunofluorescent images of postnatal day 21 pancreata. Sections are labeled with antibodies against amylase (red) for acinar cells and YFP (green) for expression of the *Rosa26^{sl-YFP}* reporter of Cre activity. Colabeled with DAPI (blue, faint) for nuclei. Genotype and treatment group are indicated above images. No groups were administered tamoxifen at any time. Column 1 shows no YFP expression in untreated mice lacking the *Ela1-Cre^{ER}* transgene (3/3 mice analyzed). Column 2 shows constitutive *Ela1-Cre^{ER}* activity in untreated mice (7/7 double transgenic mice analyzed). Column 3 shows constitutive *Ela1-Cre^{ER}* activity in spite of ivermectin treatment (2/6 double transgenic mice analyzed) whereas column 4 shows lost constitutive *Ela1-Cre^{ER}* activity following ivermectin treatment (4/6 double transgenic mice analyzed)

reporter that were exposed to ivermectin and one pup that was exposed to selamectin. From the *Gli1-Cre^{ER}*; Ai14 line, we only obtained one pup carrying both the *Cre^{ER}* and reporter transgenes that was exposed to ivermectin. Immunofluorescent labeling showed aberrant tdTomato reporter expression in the absence of tamoxifen in both the dorsal (top) portion of the niche and within the corpus callosum of *Emx1-Cre^{ER}*; Ai14 mice treated with either an ivermectin diet or topical selamectin (Fig. 2). In the *Gli1-Cre^{ER}*; Ai14 pup, the ventral (bottom) subregion of the niche displayed aberrant tdTomato expression in the absence of tamoxifen treatment in the presence of ivermectin (Fig. 2). In all cases, unexpected tdTomato-positive cells were located within regions that would normally be targeted by the relevant transgenic *Cre^{ER}* line. Notably, these findings are consistent with those of Corbo-Rodgers et al in that ivermectin, and in this additional case selamectin, treatment can aberrantly induce *Cre^{ER}* activity in the absence of tamoxifen administration.

Discussion and conclusions

Although these studies are limited in their scope, they both indicate that the highly-related antihelminthic drugs ivermectin and selamectin can alter the tamoxifen-independent activity of *Cre^{ER}* fusion proteins in the offspring of treated dams. In the case of *Ela1-Cre^{ER}*, the expected constitutive activity was reduced or abolished. In the case of both *Emx1-Cre^{ER}* and *Gli1-Cre^{ER}*, recombinase activity was aberrantly activated in the absence of tamoxifen. Although the changes in activity of the

respective *Cre^{ER}* lines were different between these studies, the results are concerning nonetheless.

The mechanism by which maternal ivermectin or selamectin affects activity of *Cre^{ER}* recombinases in offspring is not currently understood. The only other report of a similar finding [4] did not propose a mechanism and was instead more focused on the persistent activation of the *Cre^{ER}* for many weeks after cessation of ivermectin treatment. We speculate that ivermectin or selamectin treatment results in the generation of a metabolite capable of transmission transplacentally or through maternal milk thereby activating specific *Cre^{ER}* recombinases in the offspring. Ivermectin is known to be metabolized to no less than 10 different metabolites in the liver [10] and is capable of transmission via lactation [3]. It is thus possible that one of these metabolites is capable of binding to and activating the modified estrogen receptor used in *Cre^{ER}* transgenes. Further work into the scope of ivermectin and selamectin-derived metabolites, and their cross reactivity, would be enlightening.

The observed reduction in *Ela1-Cre^{ER}* activity requires a different explanation from that of the previous examples. In order to speculate about the mechanism of reduced activity, it is important to understand the nature of the constitutive activity to begin with. We hypothesize that the *elastase* promoter drives high enough levels of expression of the *Ela1-Cre^{ER}* transgene in pancreatic acinar cells to stochastically overcome the cytoplasmic retention of the *Cre^{ER}* protein by heat shock proteins that is normally relieved by tamoxifen administration. Thus, abundance of *Cre^{ER}* protein drives translocation

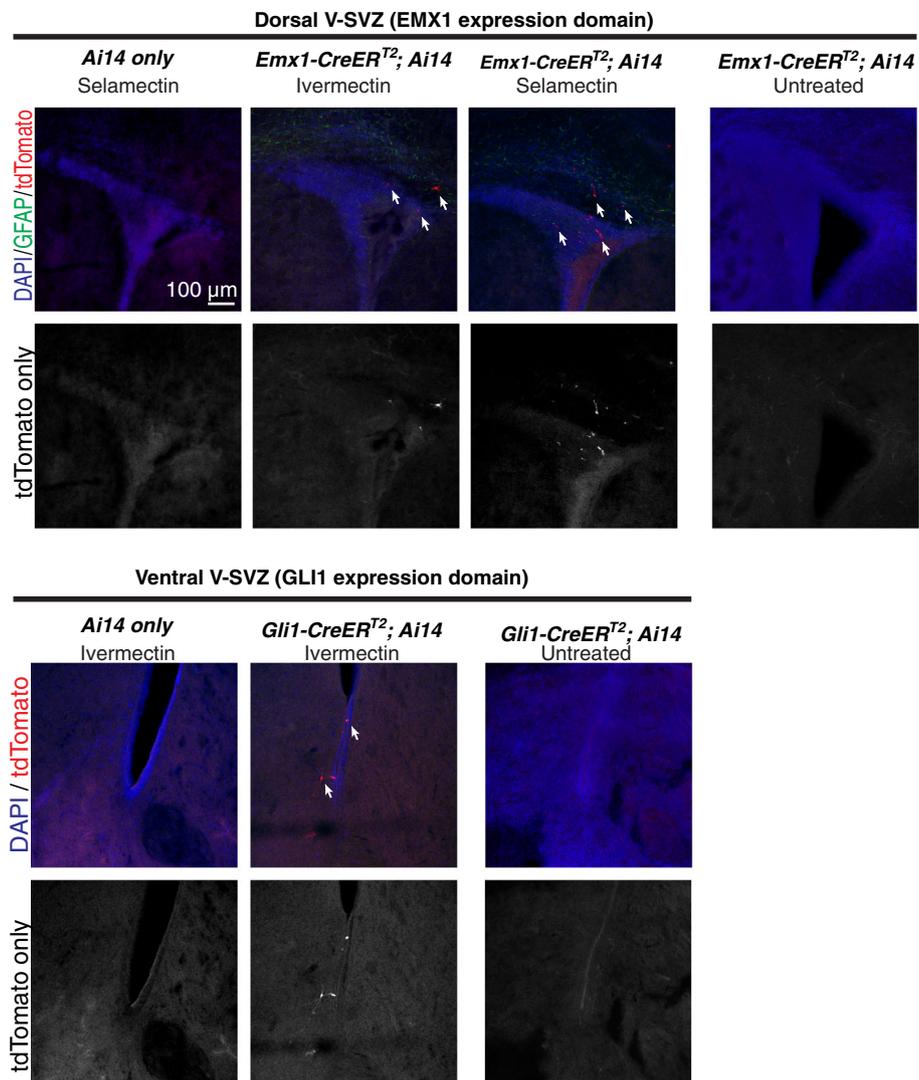


Fig. 2 Ivermectin and/or selamectin-induced activation of *Emx1-Cre^{ER}* and *Gli1-Cre^{ER}* in the V-SVZ. Representative immunofluorescent images of postnatal day 7 dorsal (above) and ventral (below) V-SVZ. Sections are labeled with antibodies against GFAP (green) for glia/stem cells and tdTomato (red) for expression of the Ai14 reporter of Cre activity. Colabeled with DAPI (blue) for nuclei. Region, genotype, and treatment group are indicated above images. No groups were administered tamoxifen at anytime. Tissue autofluorescence is visible as an artifact of imaging in control animals. Select tdTomato+ cells are indicated with arrows

into the nucleus in accordance with mass action. This hypothesis is supported by evidence that the same *Cre^{ER}* sequence driven by a *Mist1* promoter has lower expression and no such constitutive activity [8] and personal communication from Dr. Konecny). While multiple possible mechanisms could exist to reduce transgene activity, the simplest explanation for our observations is silencing of the *Ela1-Cre^{ER}* transgene as a consequence of maternal ivermectin treatment. Such silencing of transgenic elements occurs at the epigenetic level with packaging of the transgenic element into heterochromatin [2]. Anecdotally, it is thought that immune activation in the host animal leads to recognition of the transgenic

elements as “foreign” and thus the epigenetic silencing functions as a protective mechanism. Formal reports of such silencing rarely exist, but these events are well known amongst researchers using transgenic mice. In the case presented here, we hypothesize that a heightened immune response due to ivermectin treatment resulted in transgene silencing in approximately 2/3 of the offspring of treated dams. While we did attempt to measure Cre protein expression and localization with immunofluorescence, these experiments were unsuccessful at reliably labeling the Cre protein (data not shown). Importantly, *Ela1-Cre^{ER}* activity was reduced or absent in 4/9 mice born to a mother treated with ivermectin

whereas no such reduction in activity was observed in untreated animals.

Regardless of the mechanisms by which ivermectin and selamectin affect Cre^{ER} recombinase or transgene expression, the studies presented herein highlight the importance of assessing transgenic lines following treatment with either of these agents. We hope that this information is useful to the field and invites special consideration should use of a Cre^{ER} coincide with a treatment regime of ivermectin or selamectin in other labs.

Abbreviations

ER: Estrogen receptor; Ela1-Cre^{ER}: Elastase1-Cre^{ER}; Isl-YFP: lox-stop-lox-Yellow Fluorescent Protein; YFP: Yellow Fluorescent Protein; V-SVZ: Ventricular-Subventricular Zone; Emx1-Cre^{ER}: Empty spiracles homeobox 1-Cre^{ER}; Gli1-Cre^{ER}: GLI family zinc finger 1-Cre^{ER}

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Authors' contributions

P.A.K. designed, performed, and analyzed experiments as well as wrote and edited the manuscript. G.V.R. and A.A.B. designed, performed, and analyzed experiments as well as wrote and edited the manuscript. E.N.Y. provided reagents and edited the manuscript. R.A.I. and M.G. oversaw all experiments and edited the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

All protocols were approved by Vanderbilt University Medical Center Institutional Animal Care and Use Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no financial conflict of interests to publish these results.

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References

- Ahn S, Joyner AL. Dynamic changes in the response of cells to positive hedgehog signaling during mouse limb patterning. *Cell*. 2004;118(4):505–16.
- Calero-Nieto FJ, Bert AG, Cockerill PN. Transcription-dependent silencing of inducible convergent transgenes in transgenic mice. *Epigenet Chromatin*. 2010;3(1):3.
- Canga AG, Prieto AMS, Liébana MJD, Martínez NF, Vega MS, Vieitez JGG. The pharmacokinetics and interactions of ivermectin in humans—a mini-review. *AAPS J*. 2008;10(1):42–6.
- Corbo-Rodgers E, Staub ES, Zou T, Smith A, Kambayashi T, Maltzman JS. Oral ivermectin as an unexpected initiator of CreT2-mediated deletion in T cells. *Nat Immunol*. 2012;13(3):197–8.
- Kessaris N, Fogarty M, Iannarelli P, Grist M, Wegner M, Richardson WD. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. *Nat Neurosci*. 2005;9(2):173–9.
- Kropp PA, Dunn JC, Carboneau BA, Stoffers DA, Gannon M. Cooperative function of Pdx1 and Oc1 in multipotent pancreatic progenitors impacts postnatal islet maturation and adaptability. *American journal of physiology. Endocrinol Metab*. 2018;314(4):E308–21.
- Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci*. 2009;13(1):133–40.
- Shi G, Zhu L, Sun Y, Bettencourt R, Damsz B, Hruban RH, et al. Loss of the acinar-restricted transcription factor Mist1 accelerates Kras-induced pancreatic intraepithelial neoplasia. *Gastroenterology*. 2009;136(4):1368–78.
- Srinivas S, Watanabe T, Lin C-S, Williams CM, Tanabe Y, Jessell TM, et al. Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. *BMC Dev Biol*. 2001;1(1):4.
- Zeng Z, Andrew NW, Arison BH, Luffer-Atlas D, Wang RW. Identification of cytochrome P4503A4 as the major enzyme responsible for the metabolism of ivermectin by human liver microsomes. *Xenobiotica*. 1998;28(3):313–21.

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