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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

RESEARCH LETTER

Tamoxifen-Induced Gastric Injury: Effects of Dose and Method of Administration

Tamoxifen (TX) is widely used to induce Cre recombinase-estrogen receptor fusion proteins. However, gastric toxicity resulting from parietal cell death limits the utility of Cre recombinase-estrogen receptor drivers.[1] Here, we compared dose and method of TX administration in wild-type and gastrin knockout (GKO)[2] mice to define the toxic response and role of gastrin in injury and repair.

Adult mice were injected once intraperitoneally with TX (25, 50, 100, or 200 mg/kg) or vehicle (Veh) and corpus tissue was analyzed 1 or 3 days later for parietal cells and cellular proliferation. TX-induced gastric injury was apparent in hematoxylin and eosin–stained sections, with parietal cell loss shown by H/K-ATPase-α immunostaining (Supplementary Figure 1 and Figure 1*A*). Measurement of parietal cell *Atp4a* mRNA revealed a graded response (Figure 1*B*). The lowest dose, 25 mg/kg, showed no parietal cell loss, with extensive damage observed with 100–200 mg/kg, and intermediate loss at 50 mg/kg. The proliferative response, measured by EdU incorporation, was also graded (Supplementary Figure 2*A*–*J* and Figure *1C* and *D*). Increased proliferation was observed at the 2 highest doses, suggesting that a threshold of cellular damage is required to induce proliferation. In addition, the proliferative zone expanded downward in the damaged corpus glands. There was no change in antral proliferation at any TX dose (Supplementary Figure 2*K*–*S*).

Similar to other modes of gastric injury, TX has been reported to induce expression of the stem cell marker *Cd44*.[3] We observed rapid increases in *Cd44* messenger RNA for all but the 25-mg/kg dose (Figure 1*E*).

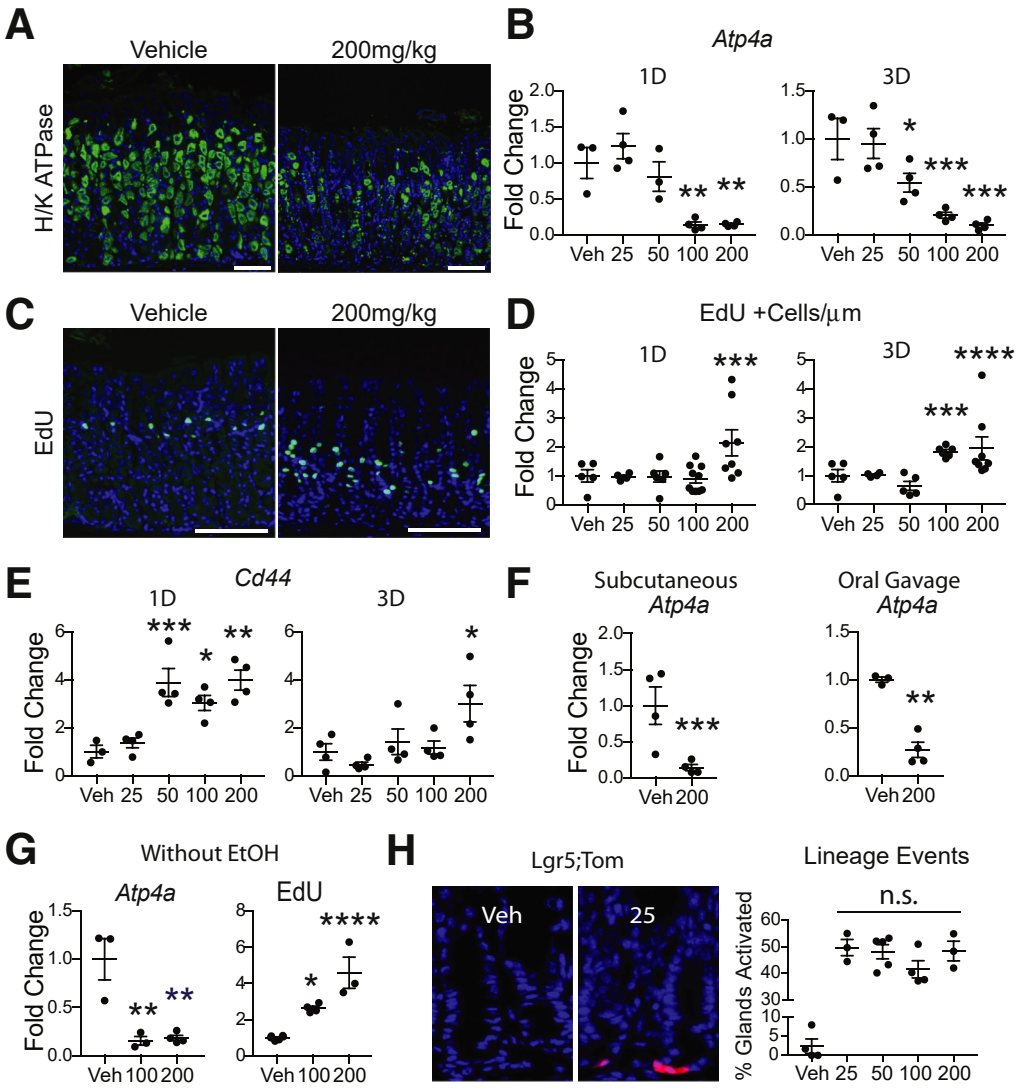

Figure 1. TX-induced gastric corpus damage is dose- and time-dependent, and route of administration and ethanol do not affect the response. (*A*) Mice treated intraperitoneally with Veh or 200-mg/kg TX were H/K-ATPase-α–immunostained (green) with DAPI (blue) at 3 days. (*B*) Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) measurement of *Atp4a* messenger RNA. (*C*) EdU staining (green) with DAPI (blue). (*A*, *C*) Scale bar = 100 μm. (*D*) EdU$^+$ cells. (*E*) RT-qPCR measurement of *Cd44* mRNA. (*F*) RT-qPCR measurement of *Atp4a* messenger RNA 3 days after 200-mg/kg TX via subcutaneous injection or oral gavage. (*G*) IP TX without EtOH at 3 days for *Atp4a* mRNA and EdU$^+$ cells. (*H*) *Lgr5-eGFP-IRES-CreERT2;ROSA-CAG-LSL-tdTomato-WPRE* mice treated with TX and examined for tdTomato reporter activation (red) 1 day later. 1D, 1 day; 3D, 3 days. Quantitative data are presented as mean ± SEM. n = 3–9 mice/treatment; *$P < .05$, **$P < .01$, ***$P < .001$, ****$P < .0001$ vs Veh by 1-way analysis of variance with Dunnett's post-test or Student's t test.

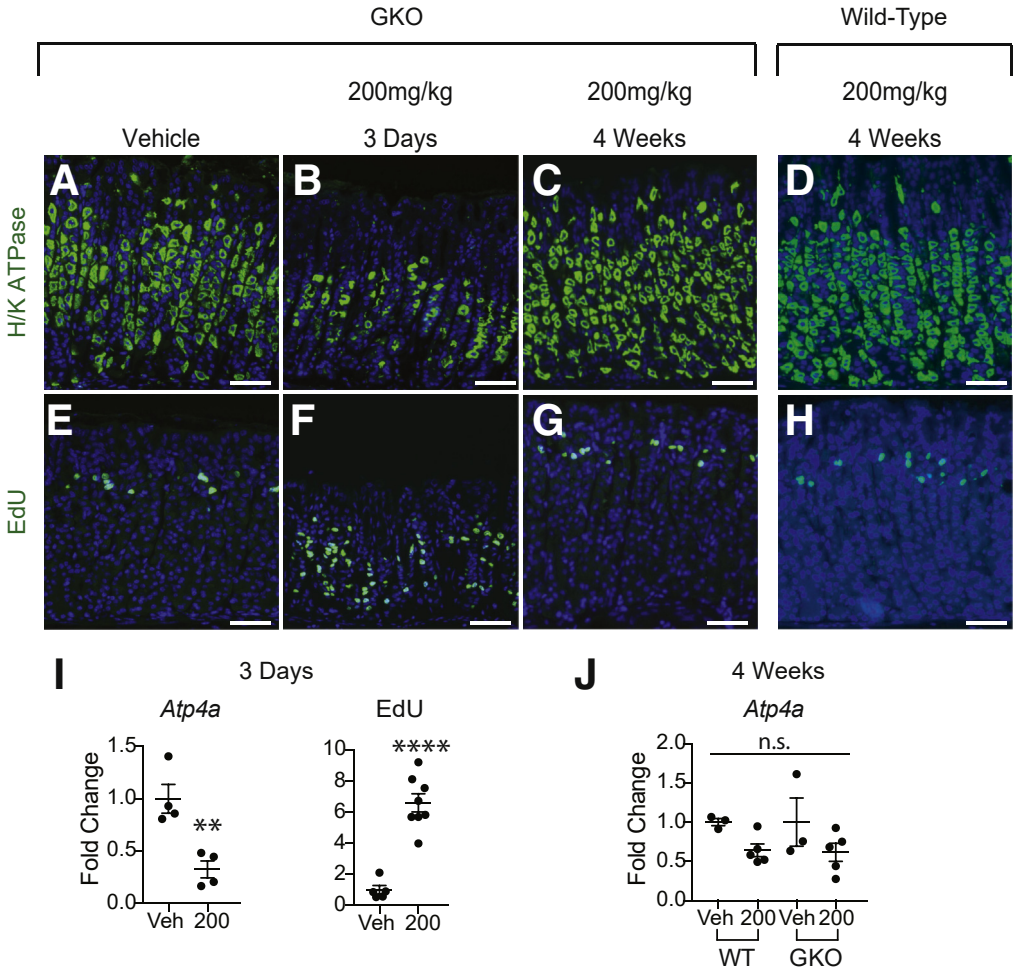

Figure 2. Gastrin is dispensable for TX damage and recovery. GKO or wild-type (WT) mice were injected intraperitoneally with 200-mg/kg TX or Veh and examined (*B*, *F*) 3 days or (*C*, *D*, *G*, *H*) 4 weeks later. Sections were stained for (*A–D*) H/K-ATPase-α (green) or (*E–H*) EdU (green), with DAPI (blue). (*I*) *Atp4a* messenger RNA abundance or EdU^{+} cells in GKO mice at 3 days and (*J*) *Atp4a* messenger RNA abundance in GKO and WT mice 4 weeks post-TX. Quantitative data are presented as mean ± SEM. $n = 3–8$ mice/treatment; $^{**}P < .01$, $^{****}P < .0001$ vs vehicle by Student's *t* test. Scale bar = 100 μm.

To determine if the mode of TX administration affects damage, mice were treated by subcutaneous injection or oral gavage, and examined for parietal cell marker expression (Supplementary Figure 3 and Figure 1*F*). As observed with intraperitoneal injection, subcutaneous injection or oral gavage induced parietal cell loss, with similar damage induced by all modes of TX administration.

To test whether the EtOH used to dissolve the TX into solution is a potential cause of the gastric toxicity, TX was made without EtOH and tissue was examined 3 days after intraperitoneal administration. This showed gastric damage, with fewer H/K-ATPase-α expressing cells (Supplementary Figure 4*A–C*), reduced *Atp4a* mRNA abundance, and increased proliferation (Figure 1*G* and Supplementary Figure 4*D–F*). The response was similar to TX with EtOH, showing that it is TX, and not EtOH, that causes gastric injury.

With the observation of a dose-dependent damage response, 25-mg/kg TX was the only dose without damage. To confirm that this dose was sufficient to induce recombination we examined lineage tracing in *Lgr5-eGFP-IRES-CreERT2;ROSA-CAG-LSL-tdTomato-WPRE* mice, comparing the extent of tdTomato-marked cells after TX activation. This analysis showed that all doses, including 25 mg/kg, induced a similar amount of labeling (Figure 1*H*). Thus, 25 mg/kg is sufficient to induce Cre recombination, and furthermore, increasing the TX dose does not increase the extent of recombination.

The gastric hormone gastrin induces corpus glandular hypertrophy and hyperproliferation in mouse genetic models with parietal cell loss or dysfunction.[4–6] To determine if gastrin is responsible for the increased epithelial proliferation observed in the corpus after TX treatment and to test the possible role of gastrin in tissue repair, GKO mice were treated with either vehicle or 200-mg/kg TX and the response was measured at 3 days to assess damage, and 4 weeks to assess restitution (Figure 2). This showed that GKO mice had a similar damage response as wild-type mice, with parietal cell loss and, importantly, markedly increased proliferation at 3 days post-TX (Figure 2*B*, *F*, and *I*). Further, gastric damage was similarly repaired in wild-type and GKO mice 4 weeks post-TX (Figure 2*C*, *D*, *G*, *H*, and *J*). Together, these data show that gastrin is not required for the TX gastric injury response or for subsequent tissue repair.

Together our findings confirm work from the Mills laboratory, which originally reported TX-induced parietal cell loss.[1,7,8] We elucidated variables that have been suggested to affect TX toxicity by systematically analyzing dose, presence or absence

of EtOH, and route of administration. Parietal cell loss and increased proliferation is a consistent feature of TX administration regardless of mode of administration or method of TX preparation. Unfortunately, many publications that use high doses of TX do not take gastric toxicity into account. As the mechanism of TX damage has not yet been uncovered, all studies that utilize TX, including both past and future studies, should consider parietal cell injury and tissue regeneration as confounding factors for any conclusions. The dynamic gastric response to TX, with rapid, yet transient changes, underscores the importance of including controls at appropriate short time points after TX administration to detect injury. Further, our studies suggest that *Cd44* messenger RNA measured 1-day post-TX would be a sensitive test of TX injury.

THERESA M. KEELEY
NOBUKATSU HORITA
LINDA C. SAMUELSON
Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan
Corresponding author: e-mail: lcsam@umich.edu; fax: (734) 936–8813.

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The authors disclose no conflicts.

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