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Tamoxifen induces rapid, reversible atrophy and metaplasia in mouse stomach

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Abstract

Tamoxifen, a selective estrogen receptor modulator, is widely used in research and clinically in patients. We find that treatment of normal mice with a single 3mg/20g body weight dose of tamoxifen leads to apoptosis of > 90% of all gastric parietal cells and metaplasia of zymogenic chief cells within 3 days. Remarkably, gastric histology returns to nearly normal by 3 weeks. Tamoxifen toxicity occurs by oral and intraperitoneal administration, in both sexes, in multiple strains, and does not depend on estrogen, though acid secretion inhibition is partially protective. Thus, substantial gastric toxicity is a heretofore unappreciated tamoxifen side effect.

Tamoxifen is widely used to spatio-temporally delete mouse genes using the CreERT/loxP system¹. Tamoxifen is also used clinically as a selective estrogen receptor (ER) modulator, in chemotherapeutic, anti-osteoporotic, and several other therapeutic regimens^{2,3}. Some reports suggest tamoxifen also increases risk for subsequent gastric cancer^{4,5}. Most gastric cancers occur in stomachs colonized by *Helicobacter pylori*⁶. Precancerous effects of bacterial colonization include: death (atrophy) of acid-secreting gastric parietal cells (PCs), differentiation changes (metaplasia) in the digestive-enzyme secreting zymogenic (chief) cell lineage (ZC) and increased stem cell proliferation^{7,8}.

In control experiments for tamoxifen induction of Cre-recombinase activity⁹, we noticed that tamoxifen injection (3 consecutive days, intraperitoneal, 5mg/20g mouse body weight) caused dramatic rearrangement of the gastric mucosa with loss of > 90% of PCs, a 6-fold increase in proliferation in stem/progenitor cells, and morphological changes in the ZCs in the bases of gastric-units (Fig. 1A-D). By 14–21 days, the epithelium recovered (Fig. 1A). No other organs had marked phenotypes at this dose or time-schedule (Supp. Fig. 1). Even a single dose of tamoxifen, by intraperitoneal injection or oral gavage, from two different commercial suppliers in three different strains of mice caused similar effects in n>63 mice (Fig. 1E, Supp. Fig. 2A-F). By qRT-PCR, PC-specific transcripts (*Atp4a*) and markers of ZC maturation (*Mist1* aka *Bhlha15*, *Pgc*, *GIF*)¹⁰ were significantly reduced by d3, whereas the surface/foveolar lineage marker (*Tff1*) and transcripts for gastrin were unchanged (Fig. 1F, see Supp. Fig. 2G for western blots of GIF and ATP4A).

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Increased progenitor cell proliferation and changes in ZC differentiation are characteristic of spasmolytic polypeptide expressing metaplasia (SPEM)^{8,11}. In SPEM, expression of mucous neck cell markers (like spasmolytic polypeptide, aka TFF2) occurs in the base of glands, where ZCs normally reside^{10,11}. Consistent with the SPEM phenotype, tamoxifen caused a marked increase in basal neck cell marker expression, (Supp. Fig. 3). Additionally, tamoxifen increased two SPEM-specific transcripts, *He4* and *Lyz*⁸; however, transcript levels for spasmolytic polypeptide (*Tff2*) itself were unchanged (Fig. 1F). SPEM is usually diagnosed by histopathological criteria and not transcriptionally^{7,8,10-12}, but we cannot rule out the possibility that the lack of increased *Tff2* indicates that tamoxifen-induced metaplasia is a SPEM variant.

In humans and mice, metaplasia always occurs in the setting of PC atrophy^{8,10}. To assess PC death, we crossed *Atp4b-Cre* mice, whose PCs constitutively express Cre¹³, to nuclear lacZ-R26R mice. In these mice, all mature PCs had, as expected, nuclear lacZ expression (Fig. 2A). Tamoxifen caused near complete loss of lacZ, indicating that PCs died and did not give rise to other cells with different morphological or molecular characteristics. TUNEL-positive PCs were not observed in the vehicle treated controls, whereas they were common within 12 hours after a single injection of 5mg/20g tamoxifen (Fig. 2B). By 12h, cytochrome C staining could now be found leaked into the cytoplasm of the majority of PCs, consistent with early apoptosis; in controls, distribution was still punctate, consistent with retention in the mitochondria (Fig. 2B). By transmission electron microscopy, PCs showed neither vacuolization nor organellar swelling, characteristics of necrotic death, but had apoptotic features like electron-dense inclusions in mitochondria and peripheral chromatin condensation (Fig. 2D, E). Finally, only tamoxifen-treated stomachs showed Caspase 3 cleavage (Fig. 2C).

Tamoxifen can function as both an estrogen receptor (ER) agonist and antagonist depending on tissue type; however, neither treatment with the pure ER agonist 17- β -estradiol nor the specific antagonist fulvestrant induced atrophy/metaplasia. And neither blocked tamoxifen effects (Supp. Fig. 4A, B). The sex of the mice also did not affect tamoxifen effects (Supp. Fig. 4C), nor did ovariectomy of females to block endogenous estrogen production (Supp. Fig. 4C; data not shown). However, tamoxifen effects could largely be reversed by blocking the PC proton pump with omeprazole (Supp. Fig. 5), suggesting a role for active acid secretion in tamoxifen toxicity to PCs. Finally, another SERM family member, raloxifene, which also has both pro and anti-estrogenic effects, did not cause atrophy up to a dose of 5mg/20g (Supp. Fig. 4D), indicating toxicity is not a general feature of SERMs. On the other hand, intraperitoneal injection of raloxifene induced Cre recombinase-mediated lacZ activation in mice expressing Rosa26-Cre fused with a modified ER (Supp. Fig. 4E), indicating that raloxifene can be used to induce Cre recombinase activity to obviate the off-target toxicity of tamoxifen in Cre-loxP inducible systems.

Tamoxifen is a chemotherapeutic drug that has toxic effects on cancer cells from diverse tissues. In osteoclasts (which, like PCs, are large, mitochondria- and proton pump-rich cells), toxicity is caused by disrupting proton gradients and, thereby intracellular pH¹⁴. The drug DMP-777 causes PC death the same way¹². Omeprazole is partially protective against both DMP-777 and tamoxifen toxicity, suggesting a similar mode of action¹². The minimal dosing that causes metaplasia in the current study is an order of magnitude more than the equivalent (40 mg/day) used therapeutically in humans¹⁵. Acute loss of PCs in patients taking tamoxifen might be beneficial for acid-reflux associated illness but could also predispose, long-term to gastric cancer. Further experiments are clearly needed to address effects of tamoxifen on the human stomach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SPEM	spasmolytic polypeptide expressing metaplasia
ZC	zymogenic (chief) cell
tEM	transmission electron microscopy
BrdU	Bromodeoxyuridine
ER	estrogen receptor
SERM	selective estrogen receptor modulator

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Fig. 1.

A) H&E of wild-type mice following intraperitoneal (i.p.) injection of vehicle at day 7 or 5mg/20g body weight tamoxifen at 3, 7, and 14 days following injection. **B)** Immunofluorescence (green: anti-ATP4A; red: anti-BrdU) at d3, quantified in **C, D, E**) Quantification of mean PCs/unit/individual mouse by H&E; unless otherwise indicated, mice were C57/B6 strain; “Tamoxifen II”: tamoxifen from another supplier. **F)** Whole stomach qRT-PCR (expressed as Log₂ scale. **P*<0.05; ***P*<0.01; ****P*<0.001)

Fig. 2.

A) Nuclear LacZ labeled PCs following tamoxifen treatment. **B) Top.** TdT-mediated dUTP nick-end labeling shows dying PCs (arrowheads). **Below:** Cytochrome C staining is punctate, consistent with mitochondrial localization in vehicle-treated (*below left*) and dispersed throughout the cytoplasm tamoxifen-treated PCs (*below right*) **C)** Cleaved caspase 3 western blot with tubulin loading control. **D)** At 2.5d following tamoxifen, PCs show chromatin condensation (arrows with black outline), consistent with early apoptosis. **E)** Another degenerating PC exhibits mitochondria ranging in morphology from normal

(dashed arrow) to electron-dense-inclusion-containing (white solid arrow) to electron-dense and degenerating (arrowheads).