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Modeling Murine Gastric Metaplasia Through Tamoxifen-Induced Acute Parietal Cell Loss

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Abstract

Parietal cell loss represents the initial step in the sequential progression toward gastric adenocarcinoma. In the setting of chronic inflammation, the expansion of the mucosal response to parietal cell loss characterizes a crucial transition en route to gastric dysplasia. Here, we detail methods for using the selective estrogen receptor modulator tamoxifen as a novel tool to rapidly and reversibly induce parietal cell loss in mice in order to study the mechanisms that underlie these pre-neoplastic events.

Keywords

Tamoxifen; Parietal cell loss; Metaplasia; Oxyntic atrophy; Spasmolytic polypeptide-expressing metaplasia (SPEM)

1 Introduction

Gastric adenocarcinoma remains one of the leading causes of cancer-related deaths worldwide [1]. The sequence of events leading to the development of gastric dysplasia and neoplasia begins with the loss of acid-secreting parietal cells, a process known as oxyntic atrophy, followed by the expansion of pre-neoplastic changes in the setting of chronic inflammation [2]. The early mucosal response to oxyntic atrophy includes reorganization of the gastric unit, characterized initially by an increased proliferation of gastric progenitor cells and the reprogramming of post-mitotic chief cells at the base of the gastric gland into a proliferating population of metaplastic cells [3]. Overall, the pattern of gastric unit reorganization that characterizes the response to oxyntic atrophy is known as spasmolytic polypeptide-expressing metaplasia (SPEM), as the metaplastic chief cells express spasmolytic polypeptide (also known as trefoil factor family 2; TFF2). SPEM can either be a transient alteration in the gastric landscape, followed by repair and restoration of normal architecture, or it can represent a crucial pre-neoplastic event en route to gastric dysplasia in the setting of chronic inflammation. The study of the mechanisms underlying the development and evolution of SPEM has been accelerated by recently developed tools [4–6] which rapidly induce SPEM in animal models of gastric dysplasia. Here, we describe the discovery and use of the selective estrogen receptor modulator, tamoxifen, as a model for studying SPEM.

In addition to its widespread therapeutic use as hormonal therapy, tamoxifen has recently found a role in conditional gene targeting in the mouse [7]. Notably, the development of a ligand-dependent Cre-ER recombinase, in which the Cre enzyme is fused to a mutated hormone-binding domain of the estrogen receptor, has allowed for the use of tamoxifen to modulate gene expression in a spatiotemporal fashion [8]. As a result, tamoxifen now serves as a tool for regulating tissue-specific Cre activity.

However, the use of tamoxifen for induction of the Cre-ER recombinase led to a serendipitous discovery in the mouse stomach that has broadened its role beyond the Cre-ER system and implicated tamoxifen as a unique agent for studying the early events following oxyntic atrophy [9, 10]. Serial intra-peritoneal injections of various strains of wild-type mice with tamoxifen induced apoptosis in the vast majority of parietal cells, metaplastic changes in the chief cells at the bases of the gastric glands, and an increased proliferation of gastric progenitor cells, changes characteristic of and consistent with SPEM. This effect is reproducible [11], estrogen-independent, and reversible, with a normalization of gastric histology within weeks of tamoxifen discontinuation [10]. The tamoxifen administration protocol described below therefore offers a unique method for reproducing oxyntic atrophy and dissecting early pre-neoplastic events leading to gastric dysplasia.

2 Materials

2.1 Preparation of Tamoxifen Stock

1. Tamoxifen (*see* Note 1).
2. Sterile sunflower seed oil (*see* Note 2).
3. Ethanol (200 proof).
4. Sonic dismembrator with microtip (2 mm).
5. Eppendorf tubes (1.5 mL).
6. Benchtop vortex machine.
7. Pipettor.
8. Protective headphones.

2.2 Mouse Injection

1. Insulin syringe with needle (0.5 mL, 27 G × 0.5 in.).
2. Balance.
3. Alcohol wipes.

[1]The source of tamoxifen has no appreciable effect on the ability to induce parietal cell loss. Tamoxifen stocks from three separate commercial suppliers, Sigma (St. Louis, MO), Cayman Chemical Company (Ann Arbor, MI), and Toronto Research Company (Toronto, Canada), have demonstrated similar efficacy [10]. In addition, parietal cell toxicity is specific to tamoxifen and not a general toxic effect of selective estrogen receptor modulators, as treatment with raloxifene, a member of the estrogen receptor modulator family with pro- and anti-estrogenic effects, had no appreciable toxicity at a comparable dose [10].

[2]To sterilize the sunflower seed oil, heat an appropriate amount in an Erlenmeyer flask on a hot plate to 85–90 °C for 15–20 min. Do not boil. Allow the flask to cool and store 40-mL aliquots at room temperature. Alternatively, the sunflower seed oil can be autoclaved prior to use.

3 Methods

Carry out all procedures at room temperature unless otherwise specified.

The following protocol corresponds to a tamoxifen solution dissolved in 10 % ethanol and 90 % sunflower seed oil (*see* Note 3).

3.1 Preparation of Tamoxifen Stock

1. Weigh mice (*see* Note 4).
2. Weigh out 25 mg of dry tamoxifen and place it in a 1.5 mL Eppendorf tube (*see* Note 5).
3. Slowly add 100 μL of 100 % ethanol, trying to keep the tamoxifen at the bottom of the tube. Do not shake, mix, or pipet.
4. Measure 900 μL of sterile sunflower seed oil in a separate 1.5 mL Eppendorf tube.
5. Sonicate the tamoxifen/ethanol mixture in the Eppendorf tube at 40 % amplitude in 20-s pulses until the tamoxifen is completely dissolved (*see* Note 6).
6. Immediately combine the tamoxifen/ethanol mixture with the sunflower seed oil. Cap and vortex the solution to ensure adequate mixing (*see* Note 7).
7. The tamoxifen mixture can be stored at 4 °C for up to 3 days or at −20 °C indefinitely (*see* Note 8). Allow the mixture to warm to room temperature prior to injection.

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[3]The free base form of tamoxifen and one of its commonly used active metabolites, 4-hydroxytamoxifen (*see* Note 12), are largely insoluble in water. The original formulation for intraperitoneal injection was found to be soluble in 60 % ethanol [12], and its solubility has since been optimized in a sunflower seed oil/ethanol mixture (*see* Note 13). Tamoxifen citrate, an oral formulation that has been developed for administering tamoxifen to mice via chow ([13]; *see* Note 10), is soluble in water at 0.3 mg/L at 20 °C. Tamoxifen-free base powder should be stored at −20 °C in the dark.

[4]Our experience has shown that three different wild-type mouse strains (*C57BL/6*, *BALB/c*, and *FVB/N*; all purchased from the Jackson Laboratory) have similar gastric mucosal responses to tamoxifen treatment [10]. In our limited experience with the strain, *BALB/c* mice are particularly sensitive to tamoxifen treatment, with mice commonly dying of unknown causes during treatment. Mice are typically used at 6–8 weeks of age, but SPEM is effectively induced in mice as old as 6 months of age. The effects on older mice are less obvious, potentially due to increased body fat causing changes in tamoxifen metabolism and distribution.

[5]An injection dose of 5 mg/20 g mouse weight over 3 days results in a dramatic phenotype, with >90 % loss of parietal cells, a significant increase in gastric progenitor cells, and morphologic changes in the chief cells at the bases of glands in the gastric corpus, histologic changes consistent with the induction of SPEM ([10], Figs. 1b and 2b). However, we have previously shown that tamoxifen injections at lower doses (≤1 mg/20 g body weight) can be used for efficient, inducible Cre-mediated recombination in the context of the Cre-ERT/*loxP* system, without the development of SPEM [9]. It is thus possible to obtain specific recombination of floxed alleles in tamoxifen-inducible Cre lines in a dose-dependent manner, while avoiding the stomach-altering effects seen at higher tamoxifen doses. Interestingly, though this has not been formally tested, SPEM induction by tamoxifen seems to have an all-or-none response, where no detectable damage can be seen at ≤1 mg/20 g mouse body weight, but ≥3 mg/20 g mouse body weight causes near complete SPEM, without an intermediate phenotype.

[6]Make sure to wear protective headphones when using the sonicator.

[7]Vortex the solution for at least 20 s. Allow the solution to sit at room temperature for several minutes. Proper mixing is crucial, and the mixture should be homogeneous. If it looks cloudy or layered, discard the mixture and start over.

[8]No appreciable decline in the ability of tamoxifen to induce SPEM has been seen for tamoxifen mixtures stored at 4 °C over the duration of injections. Similarly, the tamoxifen stock can be stored at −20 °C until further use. Our lab, however, makes a fresh tamoxifen stock prior to each treatment regimen and uses this stock for the duration of the treatment.

3.2 Tamoxifen Treatment

1. Using the insulin syringe needle, measure out the appropriate amount of the tamoxifen mixture so as to inject 5 mg tamoxifen for every 20 g mouse body weight (*see* Note 9).
2. Sanitize the injection site by wiping the mouse abdomen with an alcohol wipe. Intra-peritoneally inject the vehicle (10 % ethanol/90 % sunflower seed oil) or tamoxifen mixture (*see* Note 10).
3. Repeat the injection for 3 consecutive days using the same tamoxifen stock, stored at 4 °C.
4. Mouse stomachs can be harvested at any time following the first injection or thereafter, and tissue can be processed accordingly (Figs. 1 and 2, and *see* Note 11).

Acknowledgments

The protocol described here is based on previous studies from our lab, all performed according to protocols approved by the Washington University School of Medicine Animal Studies Committee. We acknowledge the Advanced Imaging and Tissue Analysis Core of the Washington University Digestive Disease Core Center (DDRCC) for histological preparation.

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[9]Given the viscosity of the tamoxifen mixture, aspiration into the syringe can take 10–15 s.

[10]Various tamoxifen formulations and numerous modes of tamoxifen administration have been reported. We focus here on intra-peritoneal administration, which we use most commonly, though we also observe SPEM induction with oral gavage. It is worth noting that other methods, in addition to oral gavage [14], for inducing Cre recombinase activity via tamoxifen have been used, including via drinking water [15], chow [13, 16], and subcutaneous implantation [17]. In that respect, we can only attest that oral gavage and intra-peritoneal administration of tamoxifen cause SPEM and have not tested the effects of other modes of tamoxifen administration.

[11]The effects of tamoxifen on the mouse stomach can be seen within 12–24 h of the first intra-peritoneal injection [10]. Our laboratory nomenclature designates the first day of tamoxifen injection as day 0 (D0), with the last day of injection corresponding to day 2 (D2). A recent report found that a single intra-peritoneal injection at 4 mg/25 g mouse body weight induced a 57 % loss of parietal cells in the gastric corpus [11]. In our experience, the peak effect (i.e., maximal parietal cell loss, *see* Figs. 1b and 2b) is seen at 1 day following the third tamoxifen injection (D3). We have also achieved ≥90 % loss of parietal cells at D3 even after a single injection of tamoxifen at 5 mg/20 g mouse body weight. The single-injection protocol, however, shows more variability between mice than the 3-day injection protocol. A recovery of the gastric epithelium and a return to normal histology are seen within 14–21 days [10]. Like previously described pharmacologic induction of SPEM (*see* Note 14), the effects of tamoxifen on the mouse stomach are transient. Many studies using tamoxifen-inducible Cre lines wait at least 2 weeks prior to assessing recombination, by which point parietal cells have largely recovered. This may explain how tamoxifen-induced parietal cell loss is often missed by investigators using tamoxifen to induce gene recombination in the stomach.

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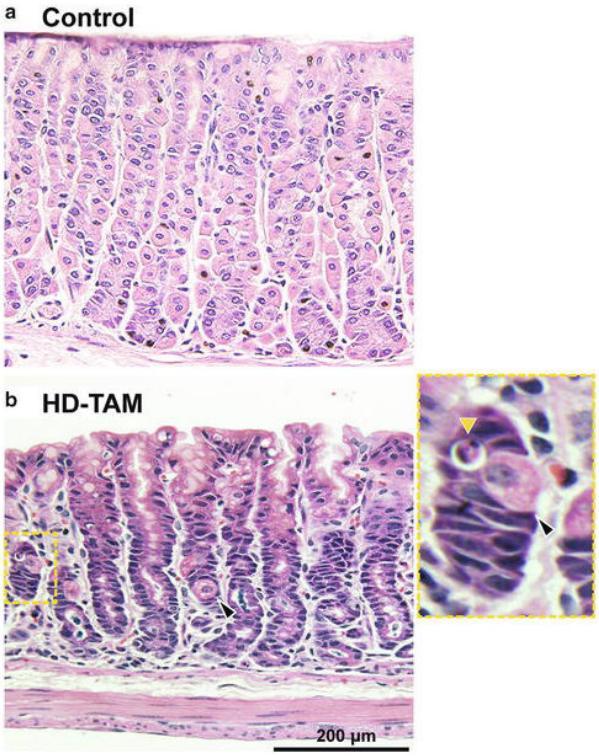

Fig. 1.

Tamoxifen treatment results in acute parietal cell loss. Representative hematoxylin and eosin stain of gastric corpus from wild-type C57BL/6 mice after intra-peritoneal injection with 3 days of either vehicle (**a**; Control) or 5 mg/20 g body weight tamoxifen (**b**; HD-TAM). Note the relative decrease in parietal cells (*black arrowhead*) compared to the vehicle-treated mouse. An apoptotic body (*yellow arrowhead*) adjacent to a dying parietal cell is highlighted (*inset*)

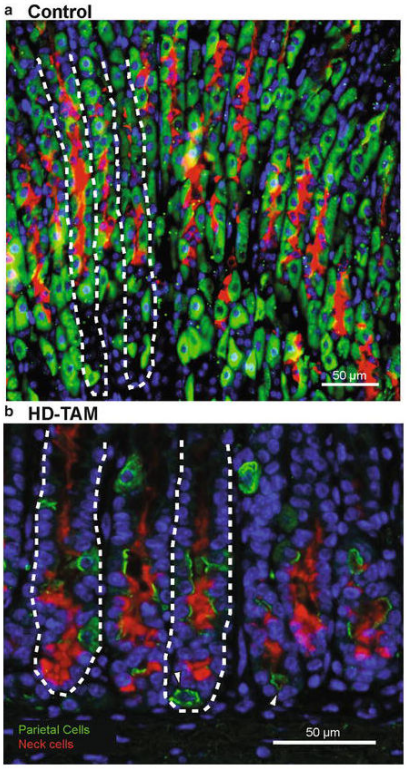

Fig. 2.

Tamoxifen treatment causes acute parietal cell loss and alters the GSII expression pattern in gastric units. (**a**) Representative immunostain of the gastric corpus of a mouse intra-peritoneally injected with vehicle alone (Control) for 3 days demonstrates normal-appearing gastric units, highlighted by abundant parietal cells (stained with H^+/K^+ ATPase; *green*) and neck cells (stained with GSII; *red*). Nuclei are stained with Hoescht (*blue*), and representative gastric units are highlighted by *dashed lines*. (**b**) A representative immunostain of the gastric corpus from a mouse intra-peritoneally injected with 5 mg/20 g body weight tamoxifen for 3 days (HD-TAM) shows an acute loss of parietal cells, as demonstrated by the relative paucity of VEGFb-staining cells (*green*). Fragments of parietal cells are highlighted by the *white arrowheads*. In addition, note the shift in GSII expression (*red*) toward the bases of glands in tamoxifen-treated mice compared to the vehicle-treated controls. Nuclei are stained with Hoescht (*blue*), and representative gastric units are highlighted by *dashed lines*

Table 1

Characteristics of various inducers of SPEM

Agent	Route of administration	Time to onset of SPEM	Inflammatory response?	Reversibility
Helicobacter pylori [a]	Oral gavage	Months	Yes	No
Helicobacter felis [a]	Oral gavage	Months	Yes	No
DMP-777	Oral gavage	10-14 days	No	Yes
L-635	Oral gavage	3 days	Yes	Yes
Tamoxifen	Intra-peritoneal, oral gavage [b]	3 days	Scant	Yes

See text and associated references for more details

[a]Reported in C57BL/6 mice and Mongolian gerbils

[b]Other forms of tamoxifen administration have been described and are referenced in the text