Subcutaneous and Intraperitoneal Tamoxifen Injections in an Outbred Rat Stock

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Introduction

Tamoxifen has been used for inducing Cre recombinase-based gene knock out in mice. Recently developed technology allows the creation of rats that carry tamoxifen-inducible Cre recombinase genes. Using this technology, a rat was genetically engineered to model human Polycystic Kidney Disease. An attempt to use a standard mouse protocol to induce Cre recombinase expression (intraperitoneal [IP] tamoxifen injection at 100mg/kg at birth and again 24 hours later) in this new model resulted in 100% mortality 5 to 7 days after injection. These unexpected results prompted the current study to determine the best dose and route of tamoxifen administration in outbred Sprague Dawley (SD) rats.

Based upon the mortality rates in the genetically engineered strain of rat, we hypothesize that:

- Neonatal rats require lower tamoxifen doses than neonatal mice
- Tamoxifen injected subcutaneously (SQ) will be absorbed more slowly than tamoxifen injected IP, leading to reduced mortality
- Decreasing doses of tamoxifen will cause proportionate decreases in mortality in litters

Methods

Treatment Group 1: Tamoxifen at 100mg/kg body weight

Treatment Group 2: Tamoxifen at 40mg/kg body weight

Treatment Group 3: Tamoxifen at 3.72mg/kg body weight

Treatment Group 4: Sunflower Oil Vehicle Only at 40mg/kg body weight

Treatment Group 5: Sham Injection, no material introduced

Injection Protocol for Adults: Injected twice, 24 hours apart. Monitored and weighed every other day for 3 weeks

Injection Protocol for Neonates: Injected within 24 hours of birth and Again 24 hours later. Monitored and weighed daily for 4 weeks

Figure 1. Treatment groups for adult animals and neonate litters. Injections were delivered either SQ or IP. Tamoxifen was dissolved in sunflower oil at 10mg/ml (Treatment Groups 1 and 2) or 1mg/ml (Treatment Group 3).

Adult Results

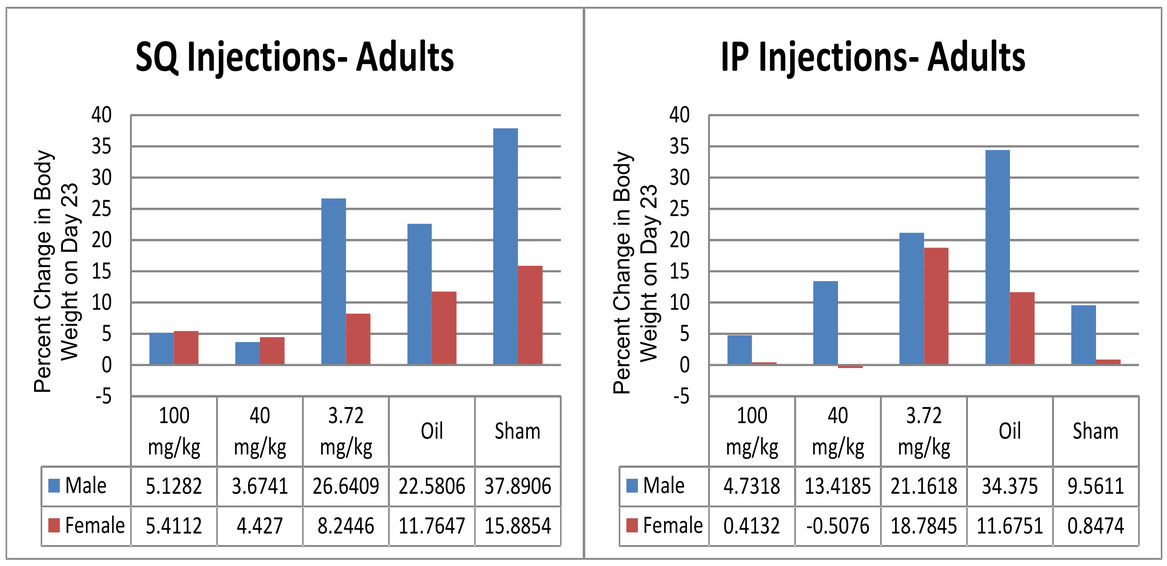

Figure 2. Graph depicting percent change in weight by treatment group (n=2, one male and one female) on the day of euthanasia, 23 days after the initial injection. All rats dosed with Tamoxifen at either 100mg/kg or 40mg/kg initially showed transient weight loss.

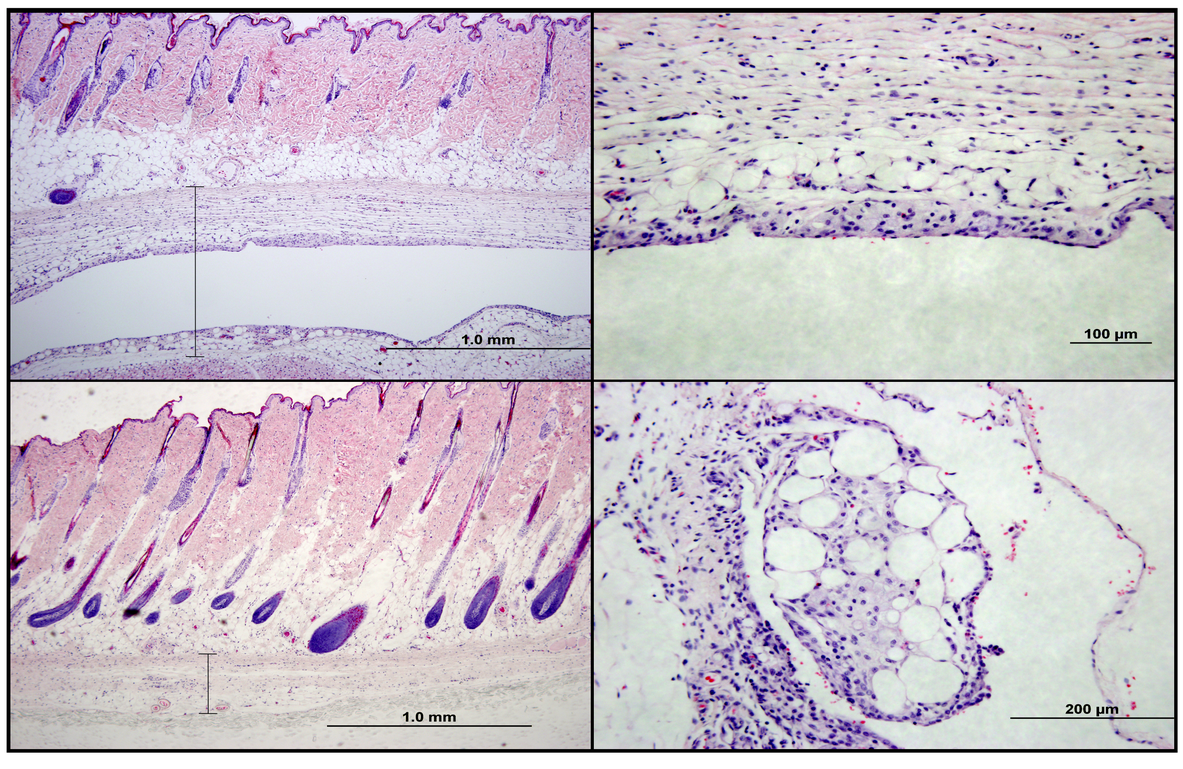

Figure 3. Histopathology of adult SD rats. (A) SQ injection region, skin with a large acellular area surrounded by macrophages. Black bracket shows difference in thickness between the SQ region of 3A and 3C. (B) Higher power of 3A showing cellular detail. (C) Skin, normal SQ control. (D) IP injection, mesentery with multiple acellular areas.

Neonate Results

Table 1. Results of neonate injections.

Treatment Group	Route of Administration	Number of Pups Born	Percent Mortality, 16 Days Old	Number of Pups With Neurological Signs	Average Weight at Birth in Grams	Average Weight, 16 Days Old in Grams
100mg/kg	SQ	8	50%	4	7.9	21.1
100mg/kg	SQ	10	100%	0	5.6	N/A
100mg/kg	IP	14	100%	0	5.9	N/A
100mg/kg	IP	15	100%	2	6.6	N/A
40mg/kg	SQ	13	0%	3	7.1	27.6
40mg/kg	IP	14	14%	0	7.1	24.9
3.72mg/kg	SQ	10	70%	0	6.9	40.1
3.72mg/kg	SQ	11	27%	0	6.6	25.8
3.72mg/kg	IP	13	0%	0	6.5	33.8
3.72mg/kg	IP	12	0%	0	7.0	33.9
Oil Only	SQ	17	71%	0	6.4	49.4
Oil Only	IP	18	39%	0	5.9	33.9
Sham	SQ	10	0%	0	7.6	35.5
Sham	IP	9	0%	0	7.0	38.0

Conclusions

- Neonates seem to tolerate the low dose of tamoxifen well, but at 100mg/kg pups surviving past 5 days displayed neurological symptoms.
- There is no significant difference between SQ or IP routes in either neonates (mortality) or adults (weight gain).
- No significant differences were found between neonate groups.

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