Importance of estradiol dosing method in mouse models of ER+ breast cancer

Jukka Rissanen[1], Leila Koskela[1], Niina Saarinen-Aaltonen[2], Merja Häkkinen[3], Seppo Auriola[3], Harri Jukarainen[1] and Saku Ruohonen[1]

[1]PreclinApps Ltd, Raisio, Finland
[2]Forendo Pharma Ltd, Turku Finland
[3]University of Eastern Finland, Kuopio, Finland

PreclinApps Ltd

INTRODUCTION

Supraphysiological 17β-estradiol (E2) supplementation supports tumor growth in estrogen receptor (ER) positive mouse models of breast cancer. In addition to conventional chronic injections, several E2 administration methods have been developed for rodents, but they all share similar problems with either adverse events or inaccurate kinetics. Most common adverse effects are bladder stone formation, hydronephrosis, urinary retention and rash. The incidence and severity of adverse effects have been associated with E2 dose, administration route, dosing method, duration of administration and mouse strain. Due to severity of these adverse effects, mice are often sacrificed prematurely leading to early termination of the experiment.

Undefined daily release and decreasing drug release rate over time are common challenges in sustained release systems. To avoid these challenges, we have developed a stable polymer-based long-term substance delivery system called the MedRod® (Figure 1), where diffusion is driven by the concentration gradient across the membrane. The technology enables stable drug release from weeks up to several years in preclinical studies.

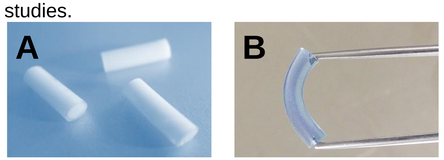

Figure 1: A) MedRod® implants intended for subcutaneous implantation for mouse or rat. B) Elastic properties of the MedRod® implants. The implants can be colored to specify different products.

AIM OF THE STUDY

In this study, we characterized the in vitro and in vivo release of E2 MedRod® implant with nominal release of 1.5 mcg/day aimed to support tumor growth of commonly used MCF-7 based ER+ breast cancer xenografts.

DRUG RELEASE OPTIONS

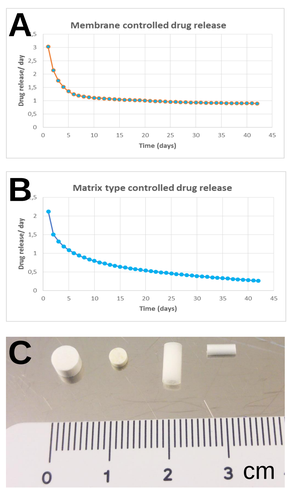

Figure 2: A) Typical release of MedRod® implants. After short initial burst, membrane controlled drug release is stable for several months. B) Typical release of matrix pellets used largely in preclinical research. Matrix controlled release typically decreases over time after initial burst. C) Different sizes of typical matrix pellets (tablets) and MedRod® systems (rods) with scalebar (cm). Different sizes are intended for different animal species and/or different dosage levels. D) Schematic structure of membrane controlled and matrix controlled implants.

Matrix pellets have been traditionally used in preclinical studies, whereas MedRod® system provides novel solution for stable compound dosing. Compared to traditionally used matrix pellets MedRod® has several benefits: 1) Drug release of MedRod® implants have been characterized in detail and the daily dose is known, 2) The release is stable even up to years. 3) They are elastic and easy to sterilize prior subcutaneous installation and 4) They can be removed at any point of study with no implant or drug residuals in animal.

MATERIALS AND METHODS

E2 was suspended in the polymer matrix and produced implant core was covered by a release rate-controlling membrane. Process resulted in elastic cylinder shaped rods where compound is safely immobilized and steadily released. Drug release is controlled by implant size, drug load and polymer materials. Several dose levels were studied in *in vitro* dissolution studies. The *in vitro* release of E2 was studied for 100 days. HPLC (1200 Agilent Technologies) with 120 EC-C18 column was used to quantify hormone levels from the dissolution samples.

E2 or placebo MedRod's® were implanted s.c. for athymic balb/c nu/nu female mice under analgesia and isoflurane anesthesia. Prior to implantation, MedRod® implants were sterilized by dipping them first into 70% ethanol, and secondly into sterile physiological saline. Approximately a 5 mm incision was made in the loose skin of the animal's neck under light anesthesia, and a pocket was bluntly dissected caudally, in which the MedRod® was gently installed using forceps. The incision was subsequently closed using absorbable sutures. HSD17B1 transfected MCF-7 cells (with corresponding growth to parental MCF-7 cell line) were inoculated s.c to both flanks. The *in vivo* release was studied for 6-weeks and the serum estrogen levels were determined by Quadropole LC-MS/MS. The tumor growth was monitored by measuring the tumor volumes by caliper. The macroscopic evaluation of adverse effects was conducted during the study and in necropsy. If needed, skin rash was treated with ointment (Vetramil®).

All animal procedures were approved by the National Animal Experiment Board of Finland. All procedures were performed according to the European Convention for the Protection of Vertebrates Used for Scientific Purposes.

IN VITRO DISSOLUTION STUDY

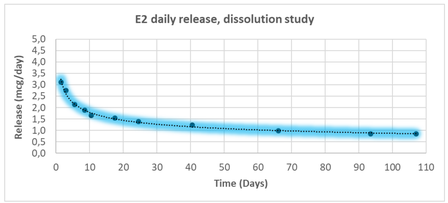

Figure 3: *In vitro* release of E2 MedRod® implants with nominal release of 1.5 mcg/day (mean + SD). After initial burst, the release was stable for 100 days. The average daily release at days 5-90 was 1.5 ± 0.5 mcg/day. The dissolution study was performed in H_2O (60 rpm at +37°C, dark). The implants were placed in vials in metal holders allowing free circulation of dissolution media.

TUMOR GROWTH AND SERUM E2

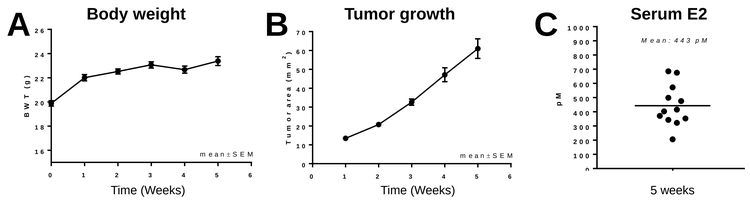

Figure 4: A) Body weight and B) tumor growth during the study after implantation of 1.5 mcg/day E2 MedRod® implants. Tumor take rate was 93% (26/28 injection sites, totally 14 mice). C) Serum E2 levels at 5 weeks after implantation of 1.5 mcg/day E2 MedRod® implants.

ADVERSE EFFECTS

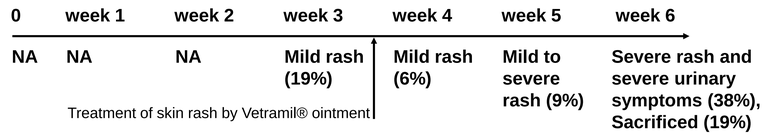

Figure 5: Occurrence of E2 related adverse effects during the study.

CONCLUSIONS

- ✓**In the *in vitro* dissolution study, the release profiles of 1.5 mcg/day E2 implants were stable for 100 days**
- ✓***In vivo*, tumor growth was constant after implantation of 1.5 mcg/day E2 implants**
- ✓**Tumor take rate was 93% demonstrating the importance of E2 supplementation in MCF-7 xenografts**
- ✓**Plasma levels of E2 were elevated during the study being at the level of 450 pM at 5 weeks after E2 supplementation**
- ✓**First signs of adverse effects were observed at week 3**
  - ✓ **Mild skin rash was observed from week 3 onwards which was partly relieved by treatment of skin ointment**
  - ✓ **At week 6 signs of urinary symptoms (enlarged bladder and urinary stones) were observed**
  - ✓ **At week 6 first animals were sacrificed due to severe E2 related adverse effects**
- ✓**All adverse effects were related to systemic supraphysiological E2 supplementation, no adverse effects were observed related to implant materials at the implantation site**
- ✓**It is extremely important to know what the correct daily dose of E2 supplement in animals is and that the dosing method is accurate and reliable**

SUMMARY

The novel MedRod® dosing system enables long-term and stable estradiol delivery in mouse models of ER+ cancer. The benefits of MedRod® system include in detailed characterized daily dose, decreased handling stress for the animals and decreased variability in results which follows closely the 3R principle (Replacement, Reduction, and Refinement). Occurrence of E2 adverse effects is dependent mainly on E2 dose and exposure time. Therefore, careful monitoring of animal health and signs of adverse effects during the study is essential in E2 supplemented preclinical cancer studies.

REFERENCES

1. Urinary retention and cystitis associated with subcutaneous estradiol pellets in female nude mice. Pearse G, Frith J, Randall KJ, Klinowska T. Toxicol Pathol. 2009 Feb;37(2):227-34.
2. Order of magnitude differences between methods for maintaining physiological 17beta-oestradiol concentrations in ovariectomized rats. Ström JO, Theodorsson E, Theodorsson A. Scand J Clin Lab Invest. 2008;68(8):814-22.