Food for Thought-the development of drug loaded diets improves both science and welfare

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EX VIVO CANCER PHARMACOLOGY
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Introduction

In our facility, we use several hormone dependent tumour models with supplementation delivered via slow release subcutaneously implanted pellets, implanted via trochar e.g.

- prostate which rely on 5-α-DHT
- breast tumours which rely on 17-β-Estradiol respectively.

Administration of E2 supplementation can result in side effects such as bladder calculi and urine scald. Therefore, to improve animal welfare and to avoid steroid supply problems, we decided to develop a new way to provide hormone supplementation via the diet.

Following on from this, we then decided to take this methodology forward into drug therapy trials.

Experiment 1

Male and female MF-1 NuNus were implanted with bioluminescent cells of LnCap and MCF-7 respectively, either subcutaneously or into the mammary fat pad and were divided into the following treatment groups:

1) Control-no supplementation
2) Supplementation via slow release pellets (IRA)
- females were implanted s.c. with 17-β-Estradiol 0.1 mg 21 day release pellets-pellets were implanted using a 10 gauge trochar (IRA, Fig. 1). (Males were not implanted with pellets due to unavailability)
3) Supplementation via diet (ssniff)
i) males were fed 5-α-DHT 2.4mg/kg (Fig 2)
ii) females were fed 17-β-Estradiol 2mg/kg (Fig 3)


Fig1-trochar and pellet


Fig 2-5-α-DHT diet


Fig 3-17-βEstradiol diet

Animals were weighed daily to ensure food was being consumed. Diets were colour coded to aid with identification.

Results 1

Tumours were measured weekly by

- calipers to calculate volumes and
- bioluminescence using the IVIS Spectrum (Perkin Elmer) to measure both tumour size and viability (Fig. 6).

The imaging results and growth curves (Figs. 6 and 10) show the supplemented diet facilitated tumour growth in both cases. With the LnCap, the increase was obvious from the start, but with the MCF-7 the effects were more marked from day 14 onwards. The MCF-7 growth data showed an increase with diet, but lower than with the pellets, while both the control groups showing little or nil growth at all.

LnCap SC
Tumour-day 35


Fig 4-no DHT

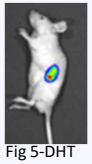
Fig 5-DHT

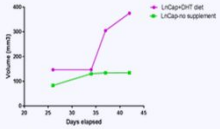

Fig 6 LnCap growth curve

MCF-7 MFP
Tumour-day 14

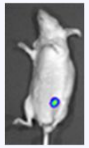
Fig 7-no E2


Fig 8-E2 pellet

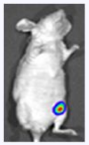
Fig 9-E2 diet

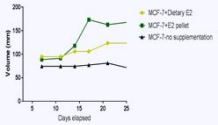

Fig 10-MCF-7 growth curve

The slower growth rate of MCF-7 could allow a longer window of opportunity for treatment effects when carrying out therapy studies. Equally importantly, we also observed a marked reduction in side effects in mice treated with the E2 dietary supplement compared with pellets, with only some slight urinary retention, which was eliminated by removing the supplemented diet for a few days, which is much easier and less invasive than removing a subcutaneous pellet.

These positive results led us to consider whether this method of delivery could also be employed in other situations. For example, there is a move towards therapeutic drugs being delivered orally, in some cases as a food supplement, so we decided to develop this methodology as an alternative to daily oral gavaging.

ACKNOWLEDGEMENTS

Thanks to ssniff Spezialdiaten GmbH and PMI TestDiet for their help and expertise in developing the diets, Prof G Seymour for allowing us to incorporate this innovation into his experiment, to Fuel3D in providing the technology and images in Expt 2 and to Marian Meakin and Alison Mackie for their technical assistance.

Experiment 2

In this experiment, a potential therapy , Substance 99, was formulated into diet and growth compared against control diet. CD-1 NuNu mice were implanted subcutaneously with HCT-116 colorectal tumour cells before receiving receive Control or Substance 99 loaded diet, with daily weights to ensure consumption.


Fig 11 Sub 99 diet

Results 2

As well as traditional caliper measurements and IVIS imaging, we also utilised the novel Fuel3D scanning system to calculate tumour volumes and record thermal images.

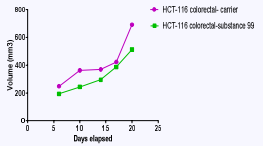

Fig 12-HCT-116 +/- Substance 99

Day 20 images

Bioluminescence Fuel3D vol. Fuel3D thermal

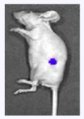
Fig 13-Sub 99

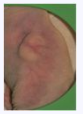
Fig 14- Sub 99

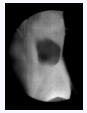
Fig 15-Sub 99

The results of this small pilot study show a slight difference between the groups with some growth delay shown in the Substance 99 treatment group We believe this demonstrates that therapies can be delivered via diet and this study gives us the confidence to pursue this method of delivery in a full scale experiment.

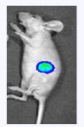
Fig 16-Carrier

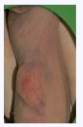
Fig 17-Carrier

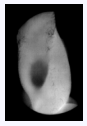
Fig 18-Carrier

CONCLUSIONS

Delivery of hormones via the diet is a major refinement in welfare terms by

- reducing the need for an invasive implant and possible removal procedure
- reducing deleterious side effects
- promoting tumour growth
- ease of delivery for the technician

Similarly, our pilot therapy experiment demonstrates that delivery of drug therapies via diet rather than daily oral gavage is both

- a welfare improvement and
- potentially a translationally more accurate model.

Therefore, we believe the potential to deliver compounds via diet rather than by more invasive techniques should always be considered wherever experimentally appropriate.