MRC Laboratory of Molecular Biology

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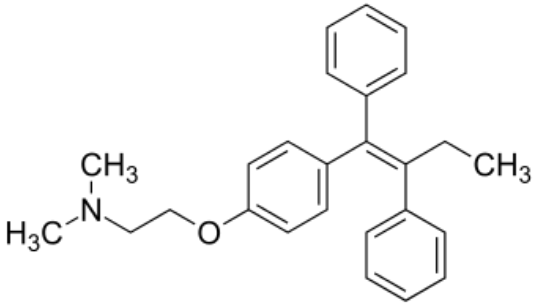
Tamoxifen Workshop 2017-Continuing a Culture of Care

A Collaborative Workshop University of Cambridge , MRC-LMB, CRUK-CI, Sanger

28th September UBS, Greenwich House, Madingley Road



Intro: Tamoxifen presentation by Trevor Littlewood

Group discussions were held around how tamoxifen is currently being delivered, the ways in which it affects the animals and how it impacts the science.

Main routes of delivery were discussed – the route depends on the experiment

- Diet – This was considered to be one of the more successful routes and is currently being used by a number of those that attended the workshop. Due to its unpalatable nature various flavours/mixes were discussed with examples such as mixing it with strawberry nesquik or using a bacon flavored variety; all with varying success rates. It was acknowledged that other edible substances needed to be removed from the cage during administration. Is better suited for long term studies.
  Refinement-Place pellets on the floor, flavoured food.
- Intraperitoneal (IP) Injection – Limited to one injection per day (alternating between sides for the injection site). Most groups who used this route mixed the tamoxifen with oil. The importance of sterilizing the oil before administration to prevent infection was discussed. There is some strain variation in how animals respond. Various oils such as peanut oil, sunflower oil and corn oil were being used with no data to suggest a certain type was more successful. This route is suitable for acute studies.
- Oral gavage – The frequency of administration varied between each group. There were very few issues reported with this method.
- Intramuscular (IM) injection – This was a less common route. One dose a day of 20 to 40 microlitres suspended in corn oil. The group using this method reported no adverse effect at the site of injection.
- Slow release pellets – Surgically implanted in the side of the neck and used to keep the tamoxifen continually in the animal's system. This was deemed to be an unsuccessful route of administration. Subcut pellet should provide circulating levels of tamoxifen which would be metabolised in the liver to the 4hydroxy metabolite.
- Topical – would need to use the more expensive 4hydroxytamoxifen metabolite of tamoxifen, otherwise it is unlikely to work. To be active tamoxifen must be metabolised by the liver andwork on the skin for tumourgenesis.
- Oral Vs Systemic- both work but if a uniform concentration is required systemic is best.

Group discussions were held around adverse effects and reactions from these routes of administration.

- Diet – Adverse effects: Scruffy/greasy coat was sometimes observed. It was acknowledged that one of the biggest issues with this route was the unpalatable nature of the mix. Once diet included the tamoxifen it was common for the mice to stop eating for up to three days afterwards; up to 30% weight loss has been encountered followed by an increase in weight when animals have started to eat again. This presented challenges when animals reached their weight loss end points and often lead to PPL amendments; the need for a different type of score sheet to combat this was identified. Going forward during their observations the technicians were looking at other factors as well as weight loss when determining end points. It is not known if the severe weight loss affects the science in other ways.
  This route was deemed unsuitable for mice less than 8 weeks of age as they would be unable to cope with the projected weight loss and the long term effects of this were unknowable. It was also acknowledged that when using tamoxifen over a longer period (such as 15-16 months) the mice suffered from curvature of the spine.
  In food, tamoxifen degrades very fast therefore pellets should be refreshed often, feeding fresh food every day will stimulate better uptake because diet exposed to light degrades and appears to become less palatable.

  Refinements – Some groups started mice on the Safe™ mash diet two or three days before tamoxifen treatment starts, to get them used to the change in texture/taste. If mash is fed, then chew sticks are required to wear teeth down.

- IP injection – Mice can get peritonitis if the oil is not sterile. Male mice may see testicular swelling, these were not always consistent and often concluded with different results. After administration there was often a small bubble of oil visible on post mortem in the area of the injection site and oil be detected in the abdomen up to six months after administration. There were also reports that animals became sick after the injection.
  There were issues identified when injecting pregnant females; this may impact the foetus, cause dystocia, there may be a need to administer progesterone to prevent abortion. The pups died or were reported as looking 'strange'/having abnormalities and cannibalism was also reported.
  There was a variation in preparation, some users re-suspend in ethanol, others sonicate, some leave in the warm overnight.

  Refinement: Tamoxifen preparation, oil should be autoclaved or filtered through 2ul filter to sterilize, the filter requires a vacuum. Once made up preparations can be frozen and withstand a freeze thaw cycle.

- Oral gavage – Problems with syringes clogging- suggested use of glass or non-reactive/stick syringes. Animals can develop loose faeces. It was noted by one group that with their four to five day dosing regimen, once the animals pass the two week point they were usually fine. This group have detected a vulnerable period at the seven to ten day post the onset of gavage where if bodyweight continues to fall animals are more likely to found dead. The exact reason for this was unclear but it was acknowledged that this could be contributed to by the technique used. If mice loose 2g/day they are most likely to die and one group euthanase mice when this weight loss is noted.

- Slow release pellets – These did not always dissolve properly and may be rejected.

- Topical – Reports of tumors growing inside the mouths of the mice due to licking the application area. Have to single house animals.

  Refinement - It was reported that when the tamoxifen was mixed with acetone it dried out the skin and creams needed to be applied to combat this. Alternatives such as mixing it with ethanol were discussed.

Group discussions were held around the main observational systems in place.

- IP Injection/diet –Weighing the mice on the day of administration was a preferred method. They would then be observed and checked at least twice a day.
  Most facilities had a variation of a 'traffic light system', 'color coding' or 'colored dots' that informed the staff of key factors such as whether there were health issues, weight loss (for example if they reached a 20% weight loss a dot would be red) or whether they had been administered the tamoxifen itself. Discussed use of microchips, weighing and spreadsheets which flag at risk animals.
  It was discussed that there was not one observational system to fit every model due to the differences in experiments being completed. A points system was not so good for endpoint recognition, a colour code system was much better.
  Diet score sheet endpoints were initially amended to limit weight loss to 20% weight loss from peak weight plus 2 other clinical signs or 25% weight loss from peak weight with no other signs.

Main points going forward –

- The diet route was an issue and needed refining. Several different mixes could be tried, mini studies undertaken and the findings shared to ensure all groups were using the most successful mix. The possibility of communicating with NC3Rs and a diet manufacturer the need for a mix that is more palatable was discussed. This would be worth implementing as a collective group rather than individually were interested. A formulation would need to be developed and one standard concentration decided upon (for the trials) and then taken forward.
  Safe™ diet plus high energy Western Road diet mix- animals loose less weight around 0-5%% verses 10-15% that can now be achieved with on normal diet. Western road diet is high in cholesterol therefore there is less weight loss but would probably not be suitable for users doing liver studies.

- Topical methods were also an issue and deemed not the most successful route of administration.

- In regards to oral gavage, sharing expertise and techniques was seen as vital for ensuring best practice. Videos reflecting this could be made and distributed. It was noted that although no training issues had been identified it was important that staff used these techniques regularly as experience was very important in ensuring the welfare of the animals involved.

- Technicians should communicate refinement methods to researchers which should facilitate good science. Researchers should know what they require and communicate this to the animal technicians.

  It was acknowledged that there was very little guidance on the route, influence of mouse strain and concentration of tamoxifen used, and scientific purpose/goal. The possibility of a survey being distributed that looked at what was done historically and how it has developed was discussed.
  Overall the need to gather this information from a wide range of sources was identified to further refine techniques and doses/concentrations used.