Linking Teaching with Research in the Disciplines

Case studies for Courses and Course teams

Teaching experimental design using research based examples

Contact details

Professor Ian Hughes, School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT

Tel: **0113-343-4313**
Fax:**0113-343-4228**
Email**: i.e.hughes@leeds.ac.uk**

Classification Category:

- *Development of student research skills*
- *Bringing data/findings from staff research into the curriculum.*

Context:

- **Course/unit/module title: Experimental Pharmacology**
- **Course title: BSc (Hon) Pharmacology**
- **Level: 3**

What does the teacher do?

The principle involved in this link between teaching and research, which can be extended to any piece of research work, is to unpick with the students the actual design process which you went through before carrying out the work, or each successive piece of work in a series. This includes the thought processes and, most importantly, where in retrospect the design was poor. The background to the problem is explained along with the real-life constraints which caused a perhaps less than ideal design to be used. The actual outcome (results) are detailed and the lessons learned.

This teaching session takes place at the start of year 3 to prepare students to design their own experiments in lab classes and research projects. The session takes one day and proceeds as follows:

Lecture on principles of experimental design (1 hour): This contains material and exemplars of good and bad practice on: the scientific method (question, hypothesis, prediction, experimental test, result and implication for hypothesis); do-ability of the designed experiment, time requirement, cost and risk associated with the experiments; consideration of data collection, accuracy and data processing (before starting the experiment); use of positive and negative controls; choice of species and numbers of repetitions; randomisation; use of blind and double blind methods; use of pilot experiments; choice of standards and comparitors; ethical experimentation, personal and professional standards and personal integrity and the 3Rs.

Exercise 1 (rest of morning) – producing a written experimental design: The class is then divided into groups of 5 and are required to produce a written design for an experiment to determine the nature of the receptors on which an unknown agonist is working to produce a contraction of the vas deferens. Start the students off by getting them to define what receptors are in the vas deferens. The importance of the scientific method is then emphasised (hypothesis, prediction, test, result and implication). The exercise takes about 35 minutes and I then start with one group and ask them what they did first. Compare with other groups and see if there are differences and explore why. Work through what they all did. What receptors are known to be in vas deferens and therefore agonist could be working on?; choose one (hypothesis) and select appropriate blocker at appropriate concentration (specificity issues); perform controls (positive and negative); test against unknown agonist. This should confirm of refute hypothesis; note possible other interpretations (e.g. indirectly acting agonist). Move to next most likely possibility, reformulate new hypothesis and repeat. Having one piece of evidence to fit your hypothesis now try a different method of confirming hypothesis (e.g. demonstrate a different drug which blocks the same receptors will also block the agonist; measure the affinity constant of the antagonist for its receptors using the unknown agonist; binding and displacement studies?).

Exercise 2 (afternoon) – producing a written experimental design which will be assessed:
The students are told to consider the problem, using a heading for each point of design which they consider and outline the issues they discussed and the conclusion they came to. This takes about 1 hour. Groups that struggle to get started can be seeded with the first two thoughts (what species; what is measured). Note that the LD50 data is just a distractor and is of very little use in determining the most effective design but some toxicity data would normally be available at this stage of a drug's development.

Exercise 2: Student Schedule

Antidepressant drugs available currently are compared on the basis of their effectiveness in relieving depression and, among other features, their cardiotoxicity after oral overdose (since depressed patients are particularly likely to take all their pills in one go in an attempt at suicide).

You are the Project Leader in a pharmaceutical firm with a current program to develop effective and less cardiotoxic antidepressant drugs. After screening 200,000 compounds for effectiveness in an excellent animal-based predictor of human efficacy your team has found 2 very effective new compounds.

You now wish to compare the 2 new compounds with existing drugs for potential cardiotoxicity in overdose before taking one or both compounds through to human studies. Unlimited amounts of all compounds are available.
There is insufficient animal data available to permit any tests in man at this time. Both drugs are effective my mouth.
The acute LD50 for the drugs is as follows:

	mg/kg i.p.	
Drug	A	B
rat	20	10
dog	50	30
rabbit	30	45

Design an appropriate experiment.

You should use me as a resource when you need information; I will not tell you how to design the experiments but will provide data to help you make choices between alternatives. There are many, many, right ways of designing this experiment.

To measure cardiac performance you may use one or all of the following:
1. electrocardiogram
2. heart rate
3. force of contraction

You are not required to present the technical details of how these parameters are determined or their relative usefulness as indicators of cardiotoxicity.

By the end of the afternoon you are required to produce and hand in a written design which will be assessed.

I then take them through the developing story of my work with mianserin at an early stage. I was initially asked to determine if the compound blocked noradrenaline uptake and chose to answer that question using isolated vas deferens and atria. When doing this it was noticed that some antidepressants changed the beating of isolated atria while others did not. To investigate this further noradrenaline uptake was measured in isolated perfused hearts at the same time as measuring the effects of antidepressants on their function. In the course of these experiments it became clear that mianserin was having less cardiotoxic effect on the heart than other antidepressants. The cardiotoxic effect was therefore examined further in in vivo experiments designed to more approximate to human antidepressant overdose. We chose to look at infusions of 4 antidepressants on cardiac performance in rabbits using the design which is reported in the papers below. Initially using conscious rabbits we found that the constant small movements interfered with the ECG recording unless a cardiac electrode was directly implanted. Because convulsions developed (another well established side effects of antidepressant overdose) these distorted the ECG records to such an extent that only initial signs of cardiotoxicity could be determined. We then repeated the experiment in anaesthetised rabbits which removed the convulsions and allowed the full cardiotoxic effects to be measured. The experiments clearly demonstrated that mianserin was less cardiotoxic in overdose that amitriptyline, impiramine or mapotiline (two reference and one competitor drug). This information was borne out in clinical use. The data presented in the papers is used for illustration including some of the original records from individual experiemnts.

Then I take them through the marking schedule for their group work and discuss each heading with the class to see what each group came up with. The marks go forward to the final assessment of the module.

Hot tips and things to look out for:
Pick one of your own papers where you designed the thing from the start, have the real original dis-agregated data, and can now see where it should have been done differently. Don't try to do this with a paper which is not yours as you will be insufficiently familiar with the details of the work.

Does it work?
My previous lectures on drug design were scored by the students at 2.8 out of 5. This session described above scored 4.6

What problems/issues have arisen?
Dealing with more than 50 students means the groups need to increase in size (I have used 7 in each group which was OK) or to get information on what they did for each design item from 3 or 4 groups and then ask if any of the other groups did it differently or had other thoughts or considerations.

Details of support material/course work/assessment methods
The schedule for exercise 2 is given above. The marking schedule is in Appendix 1.

Relevant references
B Harper & I E Hughes (1997) A comparison in isolated rabbit hearts of the dysrhythmogenic potential of amitriptyline, maprotiline and mianserin in relation to their ability to block noradrenaline uptake. British Journal of Pharmacology 59, 651-660

I E Hughes & Salwa Radwan (1979) The relative toxicity of amitriptyline, imipramine, maprotiline and mianserin in rabbits in vivo. British Journal of Pharmacology 65, 331-338

Appendix 1

Experimental design. Assessment notes.

For each point there are up to 10 marks. Decide how well you have considered the point and write the number of marks awarded on the sheet. (10 perfect; 7 first class; 4 pass)

In determining the design of your experiment you should have considered the following points.

1. What species? a. the heart should be like that of man and should be under a similar neuronal control. Rats have a high sympathetic tone, man has a high vagal tone.
b. the biotransformation of the drug in the animal should be the same as that in man; otherwise you may be looking at the cardiotoxicity of a metabolite which does not appear in man.
c. large animals need more drug; have you got an adequate supply?

2. What to measure? ECG gives a good indication of what is happening to the heart. Heart rate alone is a poor indicator as there may be changes in the muscle or the conducting tissue which do not necessarily reflect in changes in rate. Several parameters can probably be measured at the same time; e.g. HR, BP, PR interval, QRS interval, RT interval, size of R spike, nature and occurrence of any dysrhythmia. Measure a good spread of things and see what shows up in a pilot experiment.

3. Administer by what route and regimen? The oral route is the route used in self-poisoning in man and this will give a slow rise in plasma levels and some possible metabolism of the drug in the liver.
Therefore it might be best to give it orally in the animal. This would also allow for absorption to be followed by metabolism in the liver. However, oral absorption is both slow and irregular and this will introduce a lot of variability into the results.
Alternatively, give by im or sc injection however the rate of absorption from the injection site is unknown and will be variable. IV injection would give a very fast rise in plasma level and not mimic the human situation.
An alternative is slow intravenous infusion; this goes directly into the blood stream and will give a slow rise in plasma level mimicking the taking of a large oral dose. It will however bypass the first pass metabolism of the liver which would occur following oral administration. If the metabolites contribute to the cardiotoxicity this may make a difference.

4. Dose as mg or mg/kg? best to use mg/kg as this will allow for large and small animals

5. Dissolve in what vehicle? Saline is the obvious choice providing the drug will go into solution in a reasonable volume. Dimethylsulphoxide could also be used.

6. Use a control? A control group treated exactly the same as the drug groups is essential to see what changes in the heart might be produced by the vehicle or the passage of time.

7. How many animals? Difficult question until you know how variable the results are going to be. Do a few pilot experiments and then see how the data looks as you do the experiments.

8. Randomize the order of experiments? yes as your technique will change as the experiments progress and you will become better at setting up. If all one group is done first this may distort the data. Randomisation spreads changes in the animals or your technique evenly over the data.

9. Anaesthetised or conscious animals? Conscious is a better mimic of the human situation of overdose. However, cardiac changes may then be secondary to effects on the CNS. Anaesthetised animals are easier to handle but the results may be affected by the presence of the anaesthetic. This may not be important as it is a comparison between the drugs which is important.

10. Use a standard comparator drug? It is always good to demonstrate that your method will measure cardiotoxicity in an existing drug - i.e. a positive control; this also gives a comparator as to relative toxicity.

11. Which standard drug? Probably the most used in its class or the newest or the one claimed to be least cardiotoxic or the drug your new agent is likely to be in competition with.

12. How to measure an effect? You can measure
a. how big an effect is produced by a standard dose. This can cause difficulty as, say, one drug produces a 30% change and the other a 40% change in whatever is measured. Does this mean one is 10% more cardiotoxic? If you measure the size of the effect produced by a drug then you need to do this at several different dose levels so you can plot dose response curves and get a potency ratio.
b. you can measure the dose of drug required to produce a standard effect (e.g. a 20% change in some parameter. Then you can compare equitoxic doses of the two drugs and the standard.

13. What statistics? The data may be normally distributed in which case you can calculate means and standard errors. If it is not then the log of the toxic doses may be normally distributed and again means and standard errors can be calculated. Even if the data is not normally distributed means and SE can be calculated but should be interpreted with care.
If the data is normally distributed then a t-test can be used to see if the groups are statistically significantly different. Choose the appropriate formula depending on whether the variances are significantly different (determine this with a F test - variance ratio test). This would be an ordinary t-test for unpaired data since there is no basis on which to pair the experiments.
If the data is not normally distributed then non-parametric statistics should be used (e.g. a rank test) on the order in which the doses fall.

14. What will it cost? Its always good to have an idea of what expense you are incurring. It may influence the choice of method.

15. How long will it take? Its is essential to know about how long it will take to get an answer; sometimes it will make a difference to the choice of method.

16. Do a pilot experiment? Certainly. Only this will reveal if the design you have chosen will have problems. For example, only in the pilot experiments will it become

apparent that the drugs also affect the CNS and produce convulsions in conscious animals. This produces so much muscle movement that ECGs are practically impossible to measure once convulsions have started. Since they occur before serious cardiotoxicity this makes the conscious animal option less effective. In an anaesthetised animal convulsions do not occur and clear relative cardiotoxicity can be established.
Pilot experiments will also reveal the rate at which the infusion has to be conducted in order to establish cardiotoxicity within the experimental period (say 8 hours).

CONCLUSION.
This experiment will tell you about the relative cardiotoxicity of these drug UNDER THE PARTICULAR CONDITIONS OF THE EXPERIMENT. This result may or may not be the same under different conditions. It may or may not apply to man.

NOTES
It would be possible to measure the doses required in human volunteers to produce initial changes in cardiac parameters but how this would relate to cardiotoxicity in overdose is uncertain.

The definitive human data would come from overdose cases - since these are antidepressant drugs this would be forthcoming quite soon after use in the clinic.
