Use of automated blood sampling in the light of Reduction and Refinement

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Introduction

The use of automated blood sampling systems during pharmacokinetic evaluations has increased in the recent years, due to both the clear advantages this technique offers and technological improvements manufactures demonstrated.
The main advantages of repeated automated samplings could be summarized as follows:

- ***decreased animal stress (Royo F et al., 2004) and suffering with respect to other bleeding techniques (e.g., tail venopuncture);***
- ***dosing and sampling without operator's intervention, which can benefit experimental results, especially in behavioural research, and operators' wellbeing, when sample collections are required during night hours;***
- ***possibility to re-use animals, which leads to a reduction in animal use and the chance to evaluate absolute pk parameters (e.g., absolute bioavailability).***

Among the choices available on the market, the newest apparatus has been developed by DiLab and is named AccuSampler®.

Fig. 1: AccuSampler® station

Like other equipment, AccuSampler® can provide blood samples in cannulated freely moving animals, without operators' intervention.

Objective

The objective of this poster is to report data regarding the use of automated blood sampling systems in GSK Verona during 2006, to demonstrate the advantages and disadvantages this technology can offer.

Methods

Male Sprague Dawley rats were cannulated in jugular and femoral veins, to allow iv dosing and blood collection, respectively. Catheter insertion was performed in deeply Isofluorane anaesthetised animals using aseptic technique, and catheters, previously sterilised with Ethylene Oxide, were filled with a lock solution made of Glucose in heparinised saline. Flushing was performed twice a week.

Agent	Use	Dose	Route of admin.
Forane (Isofluorane)	Anaesthetic	5% (induction)	inhaled
Forane (Isofluorane)	Anaesthetic	2% (surgery)	inhaled
Rimadyl (Carprofen)	Analgesic	5 mg/kg	SC
Rubrocillina (Diidrostreptomicine + Benzilpenicilline)	Antibiotic	1 mL/rat	SC

Table 1: drugs used during surgical preparation and recovery

Animals were treated with analgesics and antibiotics on the day of surgery and let to recover for at least 3 days before use.

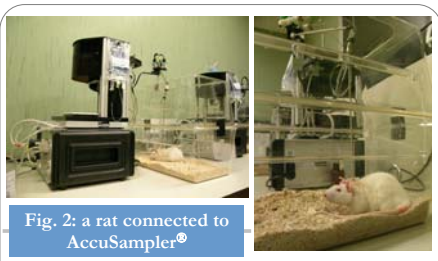

Fig. 2: a rat connected to AccuSampler®

To allow the connection of the animal to the machine, a jacket is "worn" by rats, and a swivel is attached to it during experiments.
The amount of blood collected in 24h has been calculated following indications from guidelines reported in literature (Diehl KH et al., 2001). At the end of each study, 1mL of blood from a donor rat was administered to each animal, to allow a faster recovery for the following study; washout between 2 consecutive studies consists of at least 3 days, depending mainly on the elimination phase of tested compounds.
Donor rats were those whose catheters were blocked and couldn't be (re)used.

Results

Body weight was measured after surgery, to monitor the recovery after catheter implantation. As expected, body weight slightly decreased the day after surgery but rapidly recovered (Fig. 3; data are presented as Mean ± SD, $n = 10$).

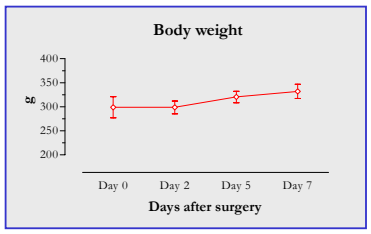

Fig. 3: Body weight of cannulated rats (Day 0 is the day of surgical preparation)

As indicators of stress and suffering during experimental procedures, **corticosterone plasma levels** were measured in animals connected to AccuSampler and compared to levels in *naïve* animals. Automatic bleedings were performed 1h post connection, and corticosterone levels (57 ng/mL, mean of 14 rats) were comparable with those obtained from decapitated rats (Marin MT et al., 2007).

As far as **Reduction** concerns, during 2006 we surgically prepared 180 rats, and performed 41 studies ($n = 6$). Considering the number of *naïve* animals ideally needed to complete the same amount of studies (41 x 6 = 246), there has been a clear Reduction of 27% (Fig. 4).

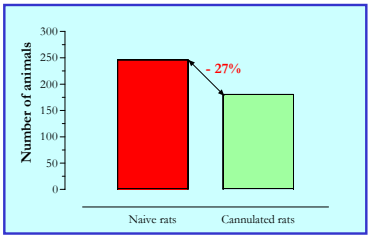

Fig. 4: Number of cannulated animals used *vs* *naïve* rats ideally needed

The possibility to perform automatic bleedings offers a clear advantage from a pk point of view: calculation of AUC and the elimination profile, for instance, can be better pictured with bleedings also during the night. Unfortunately the use of automation can cause a **dilution** of blood samples, as we observed in the pk profile of compounds tested in cannulated animals bled with AccuSampler or through manual withdrawals (Fig. 5).

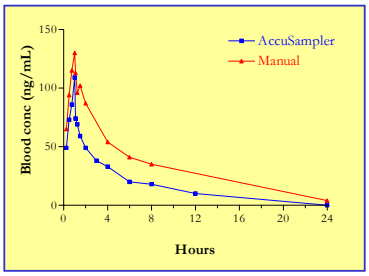

Fig. 5: Example of dilution observed *in vivo* in the pk profile of a test compound

This effect was first observed *in vitro* (data not shown), with AccuSampler taking blood from a vial containing a test compound mixed with heparinised blood, and was then experimentally confirmed *in vivo* for several compounds, giving an average of 20% dilution with respect to the profile obtained in cannulated animals but with manual bleedings. It's noteworthy to consider, anyway, that the introduction of known biological standards or the comparison of variations within the same experiment (e.g., when corticosterone increases are compared with basal levels obtained in the same experimental session) can partially counterbalance the dilution effect, giving clear scientific validity to study results.

Conclusions

Summarizing, the main disadvantages/advantages of the use of automated blood sampling are:

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- Dilution.
- Costs related to equipment and maintenance.
- Time needed to surgically prepare and regularly flush the animals.

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- Reduction.
- Increased animal wellbeing.
- Quality of data, especially when animals are regularly reused.
- Increased staff wellbeing (no nocturnal work, decreased routine, more satisfying tasks, etc.).
- Increased flexibility for study design.

When investing in these technologies it's important also to consider:

- adequate skills to perform surgery;
- facilities equipped to host aseptic surgeries;
- time needed to validate the technology and establish a reliable procedure for animal use;
- failures due to catheter patency and equipment related issues.

In our experience, a regular use of automated blood samplers allows to improve the overall performance of the studies, thus further justifying the investments needed for this technology.

References

Royo F et al., *J Endocrinol*, **180**: 145-153 (2004)
Diehl KH et al., *J Appl Toxicol*, **21**: 15-23 (2001)
Marin MT et al., *Physiol Behav*, **90**: 29-35 (2007)

All the experiments were carried out in accordance with Italian regulation governing animal welfare and protection and the European Directive 86/609/EEC, and according to internal GlaxoSmithKline Committee on Animal Research & Ethics (CARE) review.