Regulatory Toxicology and Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Clinical and anatomic pathology effects of serial blood sampling in rat toxicology studies, using conventional or microsampling methods

Alexis Caron *, Christine Lelong, T. Bartels, O. Dorchies, T. Gury, Catherine Chalier, Véronique Benning

Sanofi-Aventis Recherche & Développement, Disposition, Safety & Animal Research, Paris, France

ARTICLE INFO

Article history:
Received 9 December 2014
Available online xxxx

Keywords:
Serial blood sampling
Toxicokinetic
Rodent
Clinical pathology
Anatomic pathology

ABSTRACT

As a general practice in rodent toxicology studies, satellite animals are used for toxicokinetic determinations, because of the potential impact of serial blood sampling on toxicological endpoints. Besides toxicological and toxicokinetic determinations, blood samples obtained longitudinally from a same animal may be used for the assessment of additional parameters (e.g., metabolism, pharmacodynamics, safety biomarkers) to maximize information that can be deduced from rodents. We investigated whether removal of up to 6 × 200 µL of blood over 24 h can be applied in GLP rat toxicology studies without affecting the scientific outcome.

Methods: 8 week-old female rats (200–300 g) were dosed for up to 1 month with a standard vehicle and subjected or not (controls) to serial blood sampling for sham toxicokinetic/ancillary determinations, using miniaturized methods allowing collection of 6 × 50, 100 or 200 µL over 24 h. In-life endpoints, clinical pathology parameters and histopathology of organs sensitive to blood volume reduction were evaluated at several time points after completion of sampling.

Results: In sampled rats, minimal and reversible changes in red blood cell mass (maximally 15%) and subtle variations in liver enzymes, fibrinogen and neutrophils were not associated with any organ/tissue macroscopic or microscopic correlate.

Conclusion: Serial blood sampling (up to 6 × 200 µL over 24 h) is compatible with the assessment of standard toxicity endpoints in adult rats.

1. Introduction

Regulatory toxicity studies in animals are key components of drug development which aim at defining adverse effects of future medicines before their administration to humans. The use of rodent and non-rodent species in general toxicology is dictated by international guidelines issued by the International Conference of Harmonization (ICH, 1998, 2009). In animal toxicity studies, toxicokinetic determinations are performed to evaluate the relationship between the systemic exposure (ie, circulating concentration) of a drug and its toxicity profile (ICH, 1994). As part of common practice in toxicity studies performed in rodents, subsets of animals (satellite animals) are specifically dedicated to serial blood sampling for toxicokinetic determinations; the number of rodents used for toxicokinetics (TK) is therefore directly dependent on the number of blood sampling time points and the volume of blood samples (Sparrow et al., 2011). Best practice recommendations based on physiological limitations define the total blood volume that can be withdrawn from a single animal over a given period of time (Diehl et al., 2001).

In the rat, the rodent species the most frequently used in general toxicology, the maximal blood volume that can be withdrawn over 24 h is commonly set at 15% of the circulating blood volume (followed by a 4-week recovery period), which represents approximately 1.6 mL for a rat with 200 g of body weight (Diehl et al., 2001; Sparrow et al., 2011). Collection of this maximal volume might be required in regulatory toxicology studies to evaluate a variety of parameters such as clinical pathology, safety biomarkers, toxicokinetics or pharmacodynamic markers and combination thereof. In some cases, the total blood volume requested for the analysis of various parameters is not compatible with the maximal volume that can be withdrawn from a same animal and additional groups/subgroups of animals have to be included to achieve the study objectives. For instance, in a typical regulatory repeat-dose toxicity study, satellite groups of rats would be unavoidable to establish the toxicokinetic profile of a small molecule drug after the first and the last drug administration when the combined number and size of blood samples to be collected over 24 h exceeds the maximal volume. With this respect it is worth mentioning that the

* Corresponding author.
E-mail address: alexis.caron@sanofi.com (A. Caron).

http://dx.doi.org/10.1016/j.yrtph.2015.05.022
0273-2300/

number, frequency and size of these blood samples are determined by the pharmacokinetic properties of the drug and potentially by the sensitivity of the bioanalytical method. The specificities and the number of parameters of interest can therefore influence the design of toxicology studies as withdrawal of a relatively large volume of blood could indeed impact clinical and anatomic pathology parameters as a result of hypovolemia and finally biased the overall toxicological assessment of the drug.

Recent technological developments of bioanalytical methods have prompted the use of miniaturized blood sampling methods, which allow collection of smaller blood volumes and their use for toxicokinetic purposes. These advances include dried blood spot and, more recently, capillary microsampling methods which are amenable to diversified categories of drugs (Spreadborough et al., 2013; Dainty et al., 2012; Kaendler et al., 2013; Jonsson et al., 2012; Nilsson et al., 2013; Caron et al., 2015). In the field of toxicokinetics, a blood volume of 200 µL is commonly accepted as 'conventional sampling' and 'miniaturized sampling' can therefore be proposed for any blood sample with size $\leqslant$ 200 µL. Among these latter, there is growing acceptance that blood sample size $\leqslant$ 50 µL can be referred as 'microsampling' (Chapman et al., 2014). Routine application of miniaturized sampling methods, including microsampling, to general toxicity studies conducted in rodents can thus contribute to reduction of animal usage, by limiting/avoiding the inclusion of satellite animals solely for toxicokinetic determinations (Chapman et al., 2013). At the scale of the pharmaceutical industry, the use of main study animals for the combined assessment of toxicity and TK represents a huge benefit for the 3Rs (Reduction, Refinement, Replacement), since approximately 95% of experimental animals used in Europe are rodents (Sparrow et al., 2011). In addition, implementation of these practices is also a scientific gain since the observed toxicological effects can be correlated to the actual drug exposure in a same animal.

Consequently, the adoption of blood sampling in main study animals has become effective for the last years in rodent toxicity studies. Nevertheless, pharmaceutical companies and other stakeholders mostly reported applications in dose range finding studies rather than in regulatory (Good Laboratory Practice; GLP) studies (Chapman et al., 2014). One reason explaining this current trend is that relatively sparse data have been published to demonstrate the lack of impact of serial blood sampling on toxicologically relevant parameters. Existing publications mainly focused on clinical observations and clinical pathology parameters (Chapman et al., 2014; Caron et al., 2015) but only few reported the consequences at the histopathology level. Recently, Powles-Glover et al. investigated the toxicological effects of serial microsampling (up to 6×32 µL) in adult rats over 2 weeks (Powles-Glover et al., 2014a) or in suckling and weaned juvenile rats, and reported limited changes in hematology and splenic histopathology in weanling animals (Powles-Glover et al., 2014b).

The aim of our work was therefore to investigate the impact of serial miniaturized blood sampling in vehicle-dosed adult rats on toxicological endpoints, including clinical pathology and histopathology of selected organs. In a first study, we evaluated (i) the technical feasibility of obtaining 6 blood samples of 50 µL (i.e., microsampling), 100 µL (i.e., miniaturized sampling) or 200 µL (i.e., conventional sampling) from the saphenous vein over 24 h and (ii) the effects of these sampling procedures on standard hematology, coagulation and clinical chemistry parameters. Up to 200 µL was selected as this sample size is more likely to satisfy the need to measure a variety of parameters from a main study rat, e.g., one or several analytes for toxicokinetic purposes, but also ancillary endpoints such as pharmacodynamic (PD), safety biomarkers, etc. This study design offers the opportunity to address TK/PD relationships in a toxicity study and therefore to maximize information that can be obtained from the same animal (Chapman et al., 2013). Based on the results of the first study, a second experiment was designed (i) to follow the impact of successive blood samples (6 blood samples of 200 µL over 24 h collected from the tail vein) on clinical pathology parameters and (ii) to assess the effects of one or two toxicokinetic profiles (consisting of 6 blood samples of 200 µL over 24 h) performed 1 month apart on common parameters measured in a regulatory (GLP) 1-month oral toxicity study. Besides clinical evaluation of rats (clinical signs and body weights), assessment of standard clinical pathology parameters and histopathology of adrenal glands, bone marrow (sternum), heart, liver, spleen and thymus was performed at various time points following blood sampling session(s).

2. Material and methods

2.1. Animals, groups, housing, husbandry

Details of the study groups, blood sampling time points, and clinical and anatomic pathology evaluations performed in Studies 1 and 2 are presented in Table 1.

Table 1
Details of study groups, blood sampling time points, and clinical and anatomic pathology evaluations.

Group	Dosing duration[a]	Group size	Serial blood sampling	Clinical pathology evaluation[f]	Necropsy and anatomic pathology evaluation
Study 1					
A	1 day	$n = 9$	NA	Day 2	NA[h]
B	1 day	$n = 9$	Day 1[b,c]	Day 2	NA[h]
C	1 day	$n = 9$	Day 1[b,d]	Day 2	NA[h]
D	1 day	$n = 9$	Day 1[b,e]	Day 2	NA[h]
Study 2					
1	28 days	$n = 9$	NA	Day 29	Day 29
2	28 days	$n = 9$	Day 1[b,e,g] + Day 28[b,e,g]	Day 1[g] + Day 29	Day 29
3	9 days	$n = 9$	NA	Day 10	Day 10
4	9 days	$n = 9$	Day 1[b,e,g]	Day 1[g] + Day 10	Day 10
5	1 day	$n = 9$	NA	Day 2	NA[h]
6	1 day	$n = 9$	Day 1[b,e,g]	Day 1[g] + Day 2	NA[h]

NA: not applicable; Day 1 corresponds to the first day of dosing.
[a] Oral dosing with an aqueous solution of 0.6% (w/w) methylcellulose/0.5% (w/w) polysorbate 80 at 5 mL/kg.
[b] Blood samples for sham toxicokinetic determinations were obtained on 6 occasions (0.5, 1, 2, 4, 6 and 24 h post-dosing).
[c] Blood samples of 50 µL/time point.
[d] blood samples of 100 µL/time point.
[e] Blood samples of 200 µL/time point.
[f] Hematology, coagulation and clinical chemistry parameters.
[g] Blood samples used for hematology determinations (limited parameters; see Section 2).
[h] On study completion, rats were euthanized and discarded without further examination.

Please cite this article in press as: Caron, A., et al. Clinical and anatomic pathology effects of serial blood sampling in rat toxicology studies, using conventional or microsampling methods. Regul. Toxicol. Pharmacol. (2015), http://dx.doi.org/10.1016/j.yrtph.2015.05.022