Evaluation of Primary Hemostasis Using Bleeding Time Tests: Anatomical Sites and Species Comparison

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ABSTRACT
Drug effects on hemostasis may represent intended pharmacology or can be an observed adverse effect. Bleeding time tests (BTT) are used for diagnosis in patients but also to assess platelet function in non-clinical drug safety assessments. The goal of the study was to assess and compare anatomical sites for BTT in species commonly used for non-clinical drug development. Beagle dogs, cynomolgus monkeys, rabbits and rats were used to assess BTTs in several anatomical regions (i.e. gums, cranium, upper lip, pinna, inner cheek). Single use Surgicutt® bleeding time device for consistency in incision length, depth (1mm depth by 5mm length). Cranium presented low inter-occasion variability in all species most likely due to cutaneous attachment to the cranial structures which enables stable application of Surgicutt devices. Gums and pinna (evaluated in dogs, NHP and rabbits only) presented intermediate variability. Lips showed more significant variability possibly due to tissue immobilization differences and/or presence of varying levels of saliva. Between species, non-human primate lips (lower lip 10.9 min and upper lip 7.5 min) presented the highest inter-occasion variability but cranium (5.4 min), pinna (5.4 min) and inner cheek (4.7 min) showed values within expected ranges. Results support the use of BTT assessments in duplicate or triplicate at each timepoint to increase sensitivity of this assay in the context of toxicology assessments.

MATERIALS AND METHODS
- Incision making device (e.g.: Surgicutt®)
- Whatman’s disk filter paper
- Gauze
- Surgicel

PROCEDURE
After the sedation, the testing area was wiped with alcohol and chlorhexidine. Surgicutt was gently applied to perform 5 incisions – pinna, cranium, inner cheek, upper lip and inner lip. Measurements were taken every 30 seconds (+/- 3 seconds), from the time of incision up to 15 minutes and every minute (+/- 5 seconds) from 15 minutes until the cessation of bleeding or 1 hour, whichever occurred first. The bleeding time ended when the first blank measurement was observed.

RESULTS:
• Lowest standard deviation across species was for the cranial site.
• Lowest individual species variance noted for upper lip in dog then rat.
• Cynomolgus monkeys had the most individual variance, with the highest standard deviations when compared to other species but values remained within expected ranges for this assay.

DISCUSSION
• Across the species tested (i.e. dog, non-human primate and rat) the least variable incision site for bleeding time tests was the cranial site, clotting, on average within 2.8, 1.6 and 5.4 minutes for rats, dogs and cynomolgus monkeys from the time of incision, respectively. The lower variability of cranial bleeding time test could be related to the underlying structural support that may enable a more consistent incision. Bleeding time test results present intrinsic variability and sensitivity of this endpoint benefits from replicated measurements at the same timepoint to use average values for interpretation.

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