Validation data for the use of bradykinin and substance P protease activity assays with capillary blood and blood cards

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Abstract

In the associated main paper ("Labeled substance P as a neuropeptide reporter substance for enzyme activity" (http://doi.org/10.1016/j.jpba.2019.112953)), substance P was shown to be a valuable neuropeptide reporter substance to monitor the protease activity of serum. The assay was developed based on the predecessor assay using bradykinin ("A vote for robustness: Monitoring serum enzyme activity by thin-layer chromatography of dabsylated bradykinin products", http://doi.org/10.1016/j.jpba.2017.06.007). Both neuropeptides are of interest in inflammation and pain research and were thus explored for use with capillary blood and blood cards (see associated MethodX paper "Neuropeptide reporter assay for serum, capillary blood and blood cards"). Here, we present validation data for the assay when sampling with blood cards as well as data on the use of fresh capillary blood.

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1. Data description

This Data-in-Brief article is associated with a publication where the use of dabsylated substance P (DSP) as a neuropeptide reporter substance (NRS) for the monitoring of protease activity (PA) in serum is demonstrated (see Ref. [1] and all references therein). In an earlier work, bradykinin was used for the same purpose (http://doi.org/10.1016/j.jpba.2017.06.007). The degradation of dabsylated bradykinin (DBK) visualized the lowered protease activity in patients suffering from chronic pain. Both bradykinin and substance P are of interest in inflammation and pain research. The assay moreover requires very little sample volume (<10 μl) so that the use of blood cards was evaluated. These simplify blood collection and enable outpatient sampling.

The assay is based on the incubation of NRS with blood or serum and the detection of the generated enzymatic fragments by thin-layer chromatography (TLC). The complete protocol is available in the associated MethodX article “Neuropeptide reporter assay for serum, capillary blood and blood cards”. Brieﬂy, capillary blood was drawn and incubated with dabsylated bradykinin and substance P, respectively. Two major fragments were monitored in each case. They were separated using thin-layer chromatography (TLC). TLC-plates were scanned and the spot intensities were analysed.

For DBK, the fragments DBK1-8 and DBK1-5 were monitored as described. In healthy probands, 70–90% of DBK were degraded by 1 μl equivalent of extracted dried blood in 1 h (Fig. 1). Degradation time was shorter, when more blood equivalent was used.

In order to exclude variation due to hormonal changes, the variation in male volunteers was studied (Figs. 2–4). Standard deviation both on a single TLC-plate and on several plates was <0.03. Variation
increased with storage time of the blood card. Storage at room temperature for more than three days did not only increase the measurement error it also lowered the protease activity in dried blood. The standard deviation for 36 cooled samples of DBK1-5 of the same person was 0.09; it was even lower for the other parameters.

Fig. 3 shows experiments with three replicates each on a single TLC-plate (storage at $-20\,^{\circ}\mathrm{C}$). Sampling was performed on different days. Between-day variation was expectedly larger than inner-day variation, technical variation was smaller than biological variation.

In order to test biological variation, blood cards with capillary blood from both male and female volunteers were run in 1 h experiments. Three replicates each were examined on one TLC plate (Figs. 5 and 6; Table 1).

Most evaluation experiments with blood cards have been performed using DBK so far. An initial test for K3DSP is shown in Fig. 7.

2. Experimental design, materials, and methods

Comprehensive description of experimental protocol is available in the associated MethodX article “Neuropeptide reporter assay for serum, capillary blood and blood cards”. Briefly, capillary blood was drawn from healthy volunteers (observing the declaration of Helsinki) and incubated with dabsylated
Fig. 2. Replicate experiments with blood cards. Healthy male volunteer (29 years old). Left panel: Storage of one sample for up to 13 days. Right panel: Storage of blood cards at −20 °C or at room temperature (red). Relative Values.
bradykinin and substance P, respectively (Peptide Specialty Laboratories, Heidelberg, Germany). Two major enzymatic fragments were monitored in each case. They were separated using thin-layer chromatography (TLC). TLC-plates were scanned and the spot intensities were analysed. One microliter of capillary blood was sufficient. The incubation proceeded at 37 °C for 1 h. The TLC mobile phase was CHCl₃/methanol/H₂O/CH₃COOH, 11 : 4 : 0.6 : 0.09 v/v/v/v. For scanning a regular office scanner was

Fig. 3. Replicate experiments (12 × 3) with blood cards. Healthy male volunteer (29 y). Experiments were performed on different days on different TLC-plates. Sampling was performed on different days. Relative values.

Fig. 4. Replicate experiments with blood cards (healthy males, top: 29 y, bottom: 34 y). Relative values versus storage time in days. Standard deviations were between 0.002 und 0.044 (day 13, top) and 0.005 und 0.030 (bottom panel).
Fig. 5. Box plots of experiments with blood cards. Eight volunteers (male and female, Table 1).
Fig. 6. Replicate experiments with blood cards. Healthy volunteers (6 female, 2 male, bottom: zoom). Storage at room temperature in red. Age range 23–54 y (increasing from left).
used (Canon 9000F Mark2). Images were analysed using Photoshop plug-in Silver Efex Pro (Google, Mountain View, USA) and JustTLC (Sweday, Sodra Sandby, Sweden).

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.104873.

Reference

[1] U. Schreiber, C. Engl, M. Bayer, S. König, Labeled substance P as a neuropeptide reporter for enzyme activity, J. Pharm. Biomed. Anal. (2019), https://doi.org/10.1016/j.jpba.2019.112953.