Short-term effects of storage time and temperature on pH, pCO_2, and pO_2 in porcine arterial blood

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Summary
There is evidence that pre-analytical handling may be an important determinant of blood gas variables. To study this possibility we investigated the influence of storage time (5, 15, 30, 45, and 60 minutes after blood sampling) and storage temperature (4°C and 20°C) on the variation in pH, pCO_2, and pO_2 in porcine blood. We found that the median pH decreased ($P<0.001$), but did not exhibit clinically significant changes. The median pCO_2 increased with duration of storage ($P<0.001$) and the median pO_2 was variable at 4°C ($P=0.002$), and decreased at 20°C ($P<0.001$). The variations in pCO_2 and pO_2 were higher at 20°C than at 4°C. This study demonstrates that time delay before analysis of blood gas can be a cause of increased variation, and should be minimised in order to avoid false results and to ensure correct conclusions. If a delay of more than five minutes in analysis is expected, the specimen should be placed on crushed ice.

Sammendrag
Præ-analytisk håndtering kan være en vigtig fejlkilde ved måling af blodgas. Effekten af opbevaringstid (5, 15, 30, 45 og 60 minutter efter blodprøvetagning) og opbevaringstemperatur (4°C og 20°C) på variationen i pH, pCO_2 og pO_2 blev undersøgt i griseblod. Median-værdien for pH faldt ($P<0,001$), men faldet var dog uden klinisk betydning. Median-værdien for pCO_2 steg ($P<0,001$). Median-værdien for pO_2 varierede ved 4°C ($P=0,002$) og faldt ved 20°C ($P<0,001$). Ændringerne i pCO_2 og pO_2 var generelt større ved 20°C end ved 4°C. Dette studium demonstrerer, at tidsforsinkelse før blodgas-analyse kan være en årsag til øget variation og bør derfor begrænses, for derved at kunne undgå fejlagtige resultater og for at kunne drage korrekte konklusioner. Hvis det er nødvendigt at udsætte analysen i mere end fem minutter, bør blodprøverne opbevares på knust is.

Introduction
The porcine brain has several advantages for positron emission tomography (PET) studies *(Danielsen et al., 1998; Ishizu et al., 2000; Sakoh et al., 2000; Smith et al., 2001; Dall et al., 2002)*.
These studies have been performed in anaesthetized pigs, and PET parameters are often highly sensitive to alterations in blood gases and pH, variables that are affected by the anaesthesia. The carbon-dioxide tension (pCO_2) in particular should be stable due to its potent effects on cerebral blood flow *(Ide et al., 2003)*. Therefore, it is necessary to monitor pH and blood gases at different time points during the experiments. However, pre-analytical handling may be an important determinant, if analysis is delayed. The aim of this study was to evaluate the pre-analytical effects of storage time and storage temperature on the variation in pH, pCO_2, and the oxygen tension (pO_2) in domestic pigs and to establish guidelines for optimal handling of porcine blood samples. Previously we investigated the effect of pre-analytical handling on haematological variables, and identified certain distinctions between optimal handling of human and porcine blood samples *(Olsen et al., 2001)*.

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Materials and Methods

Animals

The experiment was performed on six domestic pigs (sows, approximately 3 months, and 37-41 kg) that were used for different PET studies. The pigs were not subjected to any specific health-monitoring program. They were fed (600 g/pig) on a restricted pellet diet (DIA plus FI, DLG, Denmark) supplemented with green fodder (Grønt-piller, DLG) and iron (Grynt, DLG), water was available ad libitum. The environmental temperature was 20°C, relative humidity 51%, with no specific light cycles, and the air was changed eight times every hour.

Blood sampling

Prior to experiments, the pigs were premedicated with 750 mg ketamine (Ketalar®; 50 mg/ml, Pfizer, Denmark) and 100 mg midazolam (Dormicum®; 5 mg/ml, Roche, Denmark). The anaesthesia was maintained with isofluran (Forene®, Abbott, Sweden) in an N_2O/O_2-mixture. Blood was obtained from a 2.3 mm Cordis® catheter (Johnson & Johnson, USA) in the left femoral artery *(Svendsen & Rasmussen, 1998)*. Six 2-ml polyethylene syringe samples (PICO 50®, Radiometer, Denmark) were drawn from each pig, anticoagulated with 80 IU dry heparin. Immediately after blood collection air bubbles were removed, and the samples were randomised and stored on crushed ice (n=3; 4°C) or at room temperature (n=3; 20°C).

Blood analysis

Blood samples were analysed at time points 5 (baseline), 15, 30, 45, and 60 minutes after their collection. Prior to each analysis, samples were agitated according to instructions in the manual (Radiometer, 1996). Blood gas analyses were performed using an ABL 550 (Radiometer, Denmark) calibrated for human blood analyses. The intra-serial coefficients of variation (CV) for the variables were based on seven repeated measurements of a single porcine blood sample at time point five minutes. The CVs were 0.023 (0.3%) for pH, 0.01 kPa (0.2 %) for pCO_2 and 0.48 kPa (2.2 %) for pO_2.

Statistics

Variation over time was analysed with Friedman´s two-way analysis of variance due to the repeated observations. When significant time effects were found ($P<0.05$), the results at different time points were compared with those at the baseline using Wilcoxon´s test. The software SPSS 10.0 (SPSS Inc, USA) was used for the calculations.

Results

Tables 1-3 present median values of pH, pCO_2, and pO_2. All the baseline values were within the range of previously reported values for domestic pigs under general anesthesia *(Thielscher et al., 1994; Dersjant-Li et al., 2002)*. There were significant variations in all three variables stored at both temperatures. The median pH was decreased in samples stored at both 4°C and 20°C (Table 1). However, the median pH decreases were very small (0.1%) compared with the CV (0.3%) for the analysis. The median pCO_2 was increased at both 4°C and 20°C (Table 2). The median increases of 1.3% at 4°C (t=15 minutes) and 1.0-2.2% at 20°C were large compared with the CV (0.2%) for the pCO_2 analysis. The median pO_2 was variable at 4°C, and decreased at 20°C (Table 3). The median decrease in pO_2 was higher at 20°C (8.2-10.8%) than the variation at 4°C (3.0-3.8%). Variations in pO_2 at both temperatures were higher than the CV (2.2%).

Discussion

The pH values were significantly decreased, at both storage temperatures but did not exhibit clinically significant changes within the study period. The pH variation is similar to results of studies performed in humans *(Harsten et al., 1988; Schmidt & Muller-Plathe, 1992; Liss & Payne, 1993)* and pigs *(Assal et al., 1980; Szenci et al., 1993; van der Wal., 1981)*. The pH decrease is probably a consequence of hydrogen ion generation from anaerobic glycoly-

Table 1. Effects of storage time and temperature on pH in porcine blood samples

	Storage temperature	
Time (minutes)	4°C	20°C
5	7.438 (7.413-7.470)	7.437 (7.424-7.470)
15	7.434 (7.408-7.466)*	7.432 (7.412-7.460)*
30	7.431 (7.408-7.461)*	7.428 (7.406-7.462)*
45	7.435 (7.412-7.466)*	7.429 (7.400-7.458)*
60	7.438 (7.420-7.475)	7.429 (7.395-7.461)*
Time effect	P<0.001	P<0.001

Median values (n=3x6) (25-75dl percentiles). *different from baseline values (t = 5 minutes) (P<0.05).

Table 2. Effects of storage time and temperature on pCO2 (kPa) in porcine blood samples

	Storage temperature	
Time (minutes)	4°C	20°C
5	5.85 (5.39-6.27)	5.83 (5.41-6.16)
15	5.96 (5.45-6.34)*	5.87 (5.43-6.20)*
30	5.97 (5.56-6.26)*	5.90 (5.51-6.31)*
45	5.97 (5.51-6.35)*	5.89 (5.54-6.33)*
60	5.87 (5.49-6.16)	5.91 (5.60-6.32)*
Time effect	P<0.001	P<0.001

Median values (n=3x6) (25-75dl percentiles). *different from baseline values (t = 5 minutes) (P<0.05).

Table 3. Effects of storage time and temperature on pO2 (kPa) in porcine blood samples

	Storage temperature	
Time (minutes)	4°C	20°C
5	14.43 (11.50-15.93)	13.89 (11.62-14.60)
15	14.35 (11.91-15.87)	13.77 (11.79-14.63)
30	14.45 (12.17-16.20)*	13.62 (12.68-14.17)
45	14.31 (12.59-15.62)	12.95 (11.14-13.57)*
60	14.90 (12.26-16.01)*	11.94 (11.09-13.74)*
Time effect	P=0.002	P<0.001

Median values (n=3x6) (25-75dl percentiles). *different from baseline values (t = 5 minutes) (P<0.05).

sis. Furthermore, the decrease is in agreement with the observed statistically significant increase in pCO_2. The pCO_2 is also increased in previous studies on human and porcine blood samples stored at room temperature *(Assal et al., 1980; Harsten et al., 1988; Schmidt & Muller-Plathe, 1992)*, although in a single study pCO_2 decreased during storage both on crushed ice and at room temperature *(Liss & Payne, 1993)*. The pO_2 varied significantly at 4°C, and decreased at 20°C. The decrease in pO_2 at 20°C was probably due to oxygen consumption: storing the blood samples on crushed ice compared with room temperature slows down cell metabolism by at least a factor of ten (Radiometer manual 1996). Ryder et al. (1988) found that increasing the storage temperature significantly decreased pO_2 in human blood. However, it is more difficult to explain the variation in pO_2 in samples stored at 4°C. Air bubbles can be a contributing factor, but they were carefully removed in this study. The pO_2 also varied in human blood samples stored at 4°C *(Liss & Payne, 1993)*. The variation in pO_2 was clinically significant, especially at 20°C. In general, the variations in pH, pCO_2, and pO_2 were higher at 20°C than at 4°C.

The pigs were anaesthetized during the experiments. Nitrous oxide and halothane, used as anaesthetics, may give unreliable pO_2 results due to the influence of these anaesthetic gases on the pO_2 electrode. However, according to an internal report from Radiometer Medical AS, nitrous oxide and isoflurane have no known influence on the pO_2 electrode used in ABL 550.

Scientifically based recommendations for handling porcine blood samples prior to blood gas analysis are available *(Assal et al., 1980; van der Wal et al., 1981; Szenci et al., 1993)*. These results are based on venous blood samples taken from awake pigs with body weights of 80-180 kg. Furthermore, these studies only focus on long-term pre-analytical effects on blood gas measurements, whereas my study focuses on short-term effects. However, these conditions are not representative for most PET studies, in which the blood samples are arterial, the studies are performed on anaesthetized pigs, and the body weight is approximately 40 kg. Even with these important differences in conditions the results of my study are in agreement with the porcine recommendations. Some common recommendations are available from other species too: according to the ABL manual (Radiometer, 1996), human blood samples are suitable for analysis after storage for up to ten minutes at 20°C, and up to 45 minutes at 5°C. In animal blood pH, pCO_2, and pO_2 can be analysed after more than one hour after collection in samples stored on ice, but pO_2 analyses are only stable for 12 minutes if blood is stored at room temperature *(Haskins, 1977)*. This is in agreement with the results of my study.

The results lead to the following conclusion: The time delay before analysis of blood gas can be a cause of increased variation in physiological parameters, and should be limited as much as possible in order to achieve reliable results and ensure correct conclusions. However, if a delay in analysis of more than 5 minutes is anticipated, the specimen should be placed on crushed ice.

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