Intestinal pH, Studied with Continuous Saline Tonometry during Ischaemia and Reperfusion in the Pig

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Objective: to evaluate continuous saline tonometry for detection of progressive intestinal ischaemia and reperfusion in a porcine model.

Design: in eight anaesthetised pigs, small bowel mucosal pCO₂ was recorded by means of two identical equipments for continuous saline tonometry and a standard tonometry balloon during ischaemia and reperfusion.

Results: both systems of saline tonometry functioned stably during the four hour protocol ischaemia, although not significant until after 45 min for one of the tonometers.

Conclusion: the equipment for continuous saline tonometry has a good reactivity, an accuracy comparable with standard tonometry.

Key Words: Continuous tonometry; Induced intestinal ischaemia; pHᵢ; Pig; Saline tonometry.

Introduction

Sigmoid ischaemia and gangrene are serious and not uncommon complications after aortic aneurysm surgery.¹ Furthermore, there is evidence that colonic ischaemia may be an underlying cause for multiple organ failure developing in patients undergoing cardiac or aortic surgery, or in patients with abdominal sepsis or hypovolemia.²⁻⁴ Discovering colonic ischaemia in early and reversible phases allows for early intervention aiming at optimising nutritional flow to vital organs, evacuation of infectious material or correction of surgical emergencies.⁵,⁶ Sigmoid ischaemia may be the first sign of decreased oxygen delivery to vital organs, and its detection is important, because correction at an early stage may reduce morbidity and mortality.⁴,⁷

Gastric or intestinal saline tonometry aimed at detecting intramucosal acidosis has been used in experimental situations, and the validity of intestinal tonometry has been verified by testing against Clark electrodes.⁸ Tonometric measurement of gastric or intestinal pHᵢ has been used in clinical set-ups for research purposes, and a lower limit for normal gastric and sigmoid pHᵢ has been proposed.⁷,⁹,¹⁰ Although a clinical prognostic value of tonometric pHᵢ registration in critically ill patients has been reported in several studies, the clinical value of the method is controversial.⁵,¹¹⁻¹⁵ In order to register tonometric sigmoid pHᵢ a sigmoidoscopy must be performed to position the tonometric catheter in the sigmoid colon. Furthermore, the standard saline tonometry is time-consuming, the analysis of pCO₂ in saline solution difficult, and different blood gas analysers show low and varying precision when measuring pCO₂ in saline solutions.¹⁶ Standard tonometry does not allow continuous measurements, and the recommended time-interval between each measurement is one hour although shorter measurement intervals were used by some authors, at least in experimental situations.⁸,¹⁷

We have earlier developed a method for continuous tonometry with circulating saline in a closed system.¹⁸ The potential advantages of continuous tonometric measurement are: fast detection of decreasing pHᵢ, inter-observer independency and long-term registration with minimal labour. In two previous reports the
accuracy and long-term function of the equipment for continuous \( \text{pH}_i \) measurement was tested during stable conditions in an *in vitro* model and *in vivo* in a porcine model.\(^{18,19}\)

The aim of the present study was to validate the continuous saline \( \text{pCO}_2 \) measurement equipment for detection of induced progressive intestinal ischaemia and reperfusion in a porcine model, and to compare the obtained results with those from standard saline tonometry.

**Materials and Methods**

*Animals, treatment and ethics*

Eight Landrace pigs with a mean weight of 27.3 kg (range 22.5–30.5 kg) were used for the experiments. The pigs were fasting overnight but allowed to drink freely. The study was approved by the University Animal Experimental Ethics Committee, and the handling of the pigs was in accordance with National Institute of Health guidelines.

**Tonometry**

The standard tonometry balloon for sigmoid measurement of \( \text{pH}_i \) was used (TRIP, Sigmoid Catheter, Tonometrics Inc. MA, U.S.A.). The semipermeable balloon at the end of the catheter was filled with 2.5 ml of physiologic saline solution, and after equilibration with the surrounding mucosa, aspirated and analysed for \( \text{pCO}_2 \) in a blood gas analyser (ABL 300, Radiometer Copenhagen). To calculate the \( \text{pH}_i \), the \( \text{pCO}_2 \) in the tonometry balloon and the bicarbonate concentration in arterial blood were entered into the Henderson-Hasselbachs equation, \[ \text{pH}_i = 6.1 + \log \left( \frac{[\text{HCO}_3^{-}]}{\text{pCO}_2 \text{corr} \times 0.0307} \right), \] where \([\text{HCO}_3^{-}]\) is the bicarbonate concentration in blood and 0.0307 the solubility of \( \text{CO}_2 \) in plasma. Analysis of \( \text{pCO}_2 \) in the saline from the standard tonometry balloon was performed every hour allowing for an equilibration time of 60 min, and as recommended by the manufacturer, the first ml of the aspirated saline was discarded before analysis.

Two identical systems for continuous saline tonometry, named the red and the white tonometer, were used in each pig during the experiments. Thus, in eight pigs a total of 16 registrations with the circulating system were performed. The system has been described in detail in a previous report.\(^{18}\) In short: a standard tonometric sigmoid catheter (TRIP, Sigmoid Catheter, Tonometrics Inc. MA, U.S.A.) was modified by adding an extra tubing leading into the balloon, and thus the physiologic saline solution in the balloon could be circulated with the aid of a pump, and the \( \text{pCO}_2 \) concentration in the saline solution measured continuously in a specially constructed measurement chamber. A transcutaneous \( \text{pCO}_2 \) sensor connected to a TCM 20 monitor (Radiometer Copenhagen) was used for measurement of \( \text{pCO}_2 \) in the saline solution (Fig. 1).

**Anaesthesia and monitoring**

Anaesthesia was induced by an intramuscular injection of 400 mg ketamine (Ketalar\(^\text{R}\), Parke-Davis Scandinavia AB) and 40 mg azaperon (Stresnil\(^\text{R}\), Janssen Pharmaceutica Belgium) and maintained by an intravenous infusion of Pentobarbitalnatrium\(^\text{R}\) (Apoteksbolaget, Sweden) at a rate varying between 360–720 mg h\(^{-1}\). Tracheotomy was performed and mechanical ventilation started (Servo Ventilator System 900B, Siemens Elema, Sweden). The ventilation rate was adjusted to maintain an endtidal \( \text{pCO}_2 \) of 4.5–5.5 kPa and the animals were ventilated with 40% oxygen in air. A central venous line was inserted in the right external jugular vein into the superior vena cava for continuous measuring of the central venous pressure (CVP), blood sampling and infusion of fluids. A catheter was placed in the aorta through the right carotid artery for continuous intraarterial blood pressure measurement and analysis of arterial blood gases. Ringer’s acetate solution was given throughout the experiment at a rate of 20–30 ml kg bw\(^{-1}\) h\(^{-1}\). We tried to maintain a normal body temperature of 37.5–38.0 °C by means of a heating pad, heating lamp and covering the pig by cloth (Table 1). Heart rate (HR), mean arterial pressure (MAP), systolic blood pressure, diastolic blood pressure, CVP, rectal temperature and endtidal \( \text{pCO}_2 \) were monitored. Erytroocyte volume fraction (EVF) and blood gases were analysed hourly and urinary production was measured every hour. Blood gas analyses were performed...
on an automatic blood gas analyser (ABL 300, Radiometer Copenhagen).

**Surgical procedure**

After catheterisation, measuring of haemodynamic parameters, and verification of the depth of anaesthesia, a midline laparotomy was performed. The distal small bowel and the ileocaecal region were identified. Through a small antimesenteric enterotomy secured by a purse-string suture a standard tonometry catheter was placed in the small bowel lumen 30 cm orally of the ileocaecal valve. At 10 and 20 cm further orally the two modified tonometry catheters for continuous tonometric measurement (red and white) were placed in the small bowel by the same technique. Thus, two identical systems for continuous pH\textsubscript{i} measurement and one standard tonometer as reference were used in each pig. A urinary catheter was inserted openly into the urinary bladder. The superior mesenteric artery (SMA) was dissected at its origin from the aorta and surrounded twice by a vessel sling running through a plastic tube. By traction on the sling the SMA was occluded. The midline incision was temporarily closed by applying multiple towel forceps to the skin.

**Experimental design**

When the pig was stabilised after the surgical procedure the experiment started at time \(-1\). One hour later, at time 0, the abdominal incision was reopened, the SMA occluded and the arterial occlusion verified by the absence of palpable pulses in the SMA and the small bowel mesenteric arteries and an increasing pallor of the small bowel wall. The laparotomy incision was closed. At time 1 the laparotomy incision was reopened, the vessel sling released and reperfusion verified by the presence of palpable pulses in the SMA and the small bowel mesenteric arteries and a visible hyperaemia in the small bowel wall. The abdomen was again closed. The first two experiments were continued 1 h after reperfusion, but since the pH\textsubscript{i} did not rise to baseline level during one hour of reperfusion, in the latter six experiments the reperfusion period was prolonged to 2 h. After the protocol was completed the pigs were killed by an overdose of potassium chloride intravenously.

**Statistics**

The statistical package for SPSS 11.0 was used for all statistical analyses. For comparison of pH\textsubscript{i} values obtained by each method at times different from baseline the Mann–Whitney \(U\)-test was used. For comparison of pH\textsubscript{i} values obtained with different methods for tonometry the Wilcoxon signed ranks test was used. The mean value of all paired differences between each continuous and the standard tonometer were calculated and presented as bias, and the standard deviation of the paired mean differences as precision. A difference was regarded statistically significant if \(p < 0.05\).

**Results**

Haemodynamic parameters and blood analyses are presented in Table 1. Heart rate and MAP during the protocol are presented in Figure 2. All other parameters were not significantly changed during the experiment (Table 1).

In all animals the red and white tonometers for continuous pCO\textsubscript{2} registration and the standard tonometer recorded a fall in pH\textsubscript{i} after occlusion of the SMA and an increase after reperfusion (Fig. 3). Statistical analysis demonstrated a significant fall in pH\textsubscript{i} compared to baseline level (time 0) after one hour of SMA occlusion (time 1) for all three tonometers (Fig. 3). One hour after reperfusion (time 2) the pH\textsubscript{i} values were still significantly lower than baseline as measured by the three tonometers. The pH\textsubscript{i} values had risen closer to baseline two hours after reperfusion (time 3) as measured by all the three systems. There was no statistically significant difference between the pH\textsubscript{i} values read each hour by the red and white tonometer. The standard tonometer measured significantly lower values than the red

<table>
<thead>
<tr>
<th>Hour</th>
<th>-1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
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<tr>
<td>Number of pigs</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>115 ± 6</td>
<td>125 ± 6</td>
<td>161 ± 13</td>
<td>119 ± 7</td>
<td>115 ± 9</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>102 ± 3</td>
<td>93 ± 8</td>
<td>103 ± 9</td>
<td>73 ± 5</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>CVP (cm H\textsubscript{2}O)</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Endtidal CO\textsubscript{2} kong (kPa)</td>
<td>4.7 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>EVF (%)</td>
<td>28 ± 1</td>
<td>29 ± 1</td>
<td>32 ± 1</td>
<td>32 ± 1</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>HCO\textsubscript{3} kong (mmol/l)</td>
<td>27.6 ± 1.0</td>
<td>27.0 ± 1.0</td>
<td>25.0 ± 1.0</td>
<td>26.0 ± 0.9</td>
<td>27.7 ± 0.6</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.0 ± 0.2</td>
<td>37.9 ± 0.2</td>
<td>37.6 ± 0.3</td>
<td>37.7 ± 0.3</td>
<td>37.8 ± 0.3</td>
</tr>
</tbody>
</table>
tonometer for hour – 1 and than the white tonometer for hours 0 and 1 (Fig. 3). Bias was found to be 0.09 pH\textsubscript{i} units for the red and 0.08 pH\textsubscript{i} units for the white tonometer, and precision 0.18 and 0.11 pH\textsubscript{i} units respectively.

Looking at shorter time intervals, a fall in pH\textsubscript{i} was noted already 15 min after occlusion of the SMA, but a statistically significant fall was not registered until after 45 minutes for the red and after 15 min for white tonometer. After reperfusion pH\textsubscript{i} continued to fall for 15 min and thereafter a steady increase of the pH\textsubscript{i} values were recorded every 15 min (Fig. 3).

**Discussion**

The evaluation of continuous saline tonometry in this study was performed in an in vivo pig model in which the SMA was clamped and the corresponding mucosal ischemia monitored with continuous tonometry as well as with standard tonometry. With continuous tonometry we noticed decreasing pH\textsubscript{i} values after 15 min, but a statistically significant fall was not detected until after 45 minutes for the red and after 15 min for white tonometer. After reperfusion pH\textsubscript{i} continued to fall for 15 min and thereafter a steady increase of the pH\textsubscript{i} values were recorded every 15 min (Fig. 3).

According to Antonsson et al., a substantial increase of pH\textsubscript{i} after reperfusion of the bowel was seen within 20 min when using microelectrodes and a smaller increase was seen within 40 min with standard tonometry, well in line with the present study. The previously determined in vitro rise-time and the in vivo equilibration time were quite long, but still the reactivity of the continuous tonometers in this study seems to be good detecting changes in pH\textsubscript{i} after SMA occlusion and reperfusion within in 15 min. Automatic data sampling from the TCM 20 monitor is easy and less laborious than performing pH\textsubscript{i} measurements with short intervals with standard tonometry.

The accuracy of the standard tonometer has been validated in several reports, and in the present study the red and white continuous tonometer compared well with the standard tonometer. The standard tonometer as well as both equipments for continuous pH\textsubscript{i} measurement recorded a significant decrease in pH\textsubscript{i} after one hour of arterial mesenteric occlusion, and an increase in pH\textsubscript{i} was seen after reperfusion. The recorded mean values for the red and white tonometers were nearly identical at every hour (Fig. 3). The bias and precision were in the same range as in our previous study. The somewhat larger differences may
be explained by the lower number of paired measurements and the fact that bias and precision increased during the ischaemic phase. The standard tonometer generally registered lower values than the red and white continuous tonometers, well in line with our previous studies.\textsuperscript{19,19} In continuous saline tonometry the bias has varied from 0.04±0.09, values possible to use as an approximate correction factor if the pH\textsubscript{i} values are to be compared to those of standard saline tonometry.\textsuperscript{19} In order to use continuous saline tonometry in a clinical setting the factor needs to be clearly defined, or levels for critical pH\textsubscript{i} values for continuous saline tonometry have to be established.

Introduction of a semi-continuous automated gas analyser, the Tonocap\textsuperscript{8}, has made it possible to perform pCO\textsubscript{2} measurements with 10–20 min intervals. \textit{In vitro} the Tonocap\textsuperscript{16} has been found to be highly precise with negligible bias, but showing lower pCO\textsubscript{2} values than saline tonometry.\textsuperscript{22} In a clinical study on patients in cardiogenic shock Jannssens \textit{et al}. found a good correlation between gastric air and saline tonometry, but pCO\textsubscript{2} measured by air was found to be significantly lower than that measured by saline, and especially at high pCO\textsubscript{2} values the disagreement increased.\textsuperscript{23} Barry \textit{et al}. in a clinical study of general ICU patients demonstrated clinically significant differences in values for gastric mucosal pCO\textsubscript{2} between air and saline tonometry, but in their study air tonometry showed higher values.\textsuperscript{24} On the other hand Heinonen \textit{et al}. demonstrated a generally good agreement between gastric air and saline tonometry in a clinical study on septic patients treated in an intensive care unit.\textsuperscript{25} Reliable time interval between measurements for the Tonocap\textsuperscript{10} seems to be between 10 and 20 min.\textsuperscript{16,25,26} Another technique for continuous pCO\textsubscript{2} measurement was presented by Knichwitz \textit{et al}.; they used a specially constructed fibreoptic pCO\textsubscript{2} sensor and presented values with 30 min intervals in a study on pigs with reduced SMA blood flow.\textsuperscript{27} There is an ongoing interest in developing an easy-to-use equipment for continuous intraluminal mucosal tonometry and the role of gas and saline tonometry in this respect is not settled.

In conclusion, the present study suggests that the equipment for continuous saline tonometry has a good reactivity, an accuracy comparable with standard tonometry and the time-consuming work with standard tonometry performed with short intervals is reduced. On the other hand, the filling of saline in the tubings and measuring chamber, the handling of the equipment and the calibration of the TCM 20 monitor also need precise handling in order to achieve reliable results. We find the initial results reported in this study with continuous saline tonometry promising. Future studies will continue and reveal the value of this new technique and other techniques for continuous or semi-continuous tonometry.

\textbf{References}


Continuous Saline Tonometry During Intestinal Ischaemia


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