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Continuous intra-arterial blood pH monitoring in rabbits with acid–base disorders

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ABSTRACT

The acid–base balance of arterial blood is important for the clinical management of seriously ill patients, especially patients with acute lung injury or acute respiratory distress syndrome. We developed a novel fluorosensor for continuous blood pH monitoring and evaluated its performance both in vitro and in vivo in rabbits with acid–base disorders. The pH sensor is made of N-allyl-4-piperazinyl-1, 8-napthalimide and 2-hydroxyethyl methacrylate, which were bonded at the distal end of the optical fiber. The fluorescence intensity increased as the pH decreased with good reproducibility, selectivity and linearity in the pH range of 6–8. The pH measurement precision was 0.03 ± 0.03 pH units with a bias of -0.02 ± 0.04 ($n = 105$) and -0.00 ± 0.05 pH units ($n = 189$) in rabbits with metabolic and respiratory acid–base orders, respectively. The optical pH sensor can accurately measure pH fluctuations with a fast response and is a promising candidate for continuous in-line measurements of blood pH in critical care patients.

1. Introduction

The accurate measurement of arterial blood pH is critical in the clinical assessment of patients with respiratory and metabolic disorders, such as acute exacerbation of chronic obstructive pulmonary disease, acute lung injury, acute respiratory distress syndrome, kidney dysfunction and multi-organ failure (Dubuis et al., 2004; Iizuka and Fregosi, 2007; Jay, 2011). Currently, blood pH is measured by blood gas analysis (BGA) using a glass electrode probe. The limitations of this method include the inability to attain immediate information on pH changes and the increased likelihood of infection and blood loss upon multiple blood samplings (Mahutte, 1994).

Blood pH can change rapidly and unexpectedly in patients compromised by acute lung injury and acute respiratory distress syndrome, especially in patients on mechanical ventilation. It is necessary to continuously monitor the blood pH of these patients to allow for the timely clinical management of the condition (Ganter et al., 2007).

Recent intra-arterial blood gas monitoring systems, Paratrend 7+ and Neotrend (Diametrics Medical Inc., HighWycombe, UK), have been developed based on an absorption and reflectance mechanism for pH sensing (Morgan et al., 1999). However, these two systems have rarely been used in clinical practice and have been withdrawn from the market due to high costs, instability and inaccuracies (Ganter et al., 2007). Fluorescence-based pH sensing is one of the most widely used optical techniques (Kasik et al., 2010; Schroder et al., 2005; Yang and Wang, 2007) and is known to be advantageous over the conventional glass electrode and absorption-based pH sensors (Lin, 2000). However, most of the studies using fluorescence-based pH sensors have only been done in vitro (Borisov et al., 2009; Wencel et al., 2009). Gan et al. (2003) found that the 1, 8-naphthalimide structure is an excellent fluorophore with high stability and quantum yield (Gan et al., 2003; Georgiev et al., 2011; Li et al., 2006; Niu et al., 2004). Recently, a series of naphthalimide derivatives were synthesized and studied as intracellular and extracellular pH sensors (Tian et al., 2010).

In this study, we immobilized one of the naphthalimide derivatives on the optical fiber for the measurement of blood pH for the first time and investigated its effectiveness and accuracy in physiological solutions under varying conditions in rabbits with respiratory and metabolic acid–base disorders to evaluate the potential application of the fiber optic pH fluorosensor in clinical practice.

2. Materials and methods

The protocols used for animal care and experimental design were approved by the local Ethical Board for Animal Research of the Zhongshan Hospital of Fudan University, Shanghai, China.

2.1. Fabrication of the fiber optic pH sensor

The probe of the optical pH sensor consists of an optical fiber and a sensing film bonded covalently to the distal end of the optical

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fiber. A proton-sensitive fluorescent dye N-allyl-4-piperazinyl-1, 8-napthalimide (APN) was synthesized as previously described (Gan et al., 2003; Li et al., 2006; Niu et al., 2004). The sensing film is a sol–gel co-polymer, derived from APN and 2-hydroxyethyl methacrylate (HEMA) by heat-polymerization. The original probe was dip-coated with a solution of MPC-co-BMA in tetrahydrofuran (Patent pending no.: China 200410053454.1, USA US11/196,305 and Japan 2005-226678), which has a high biocompatibility. The optical fiber is 1 M long, while the probe is 5 mM long with an outer diameter of 0.75 mM.

2.2. Fluorescence measurements

The optical pH sensor was connected to a high-sensitivity USB2000 spectrometer (Ocean Optics, Dunedin, FL, USA) and a lamp power supply via a Y-type bifurcated optic fiber (Ocean Optics, Dunedin, FL, USA). The pH sensor was illuminated using a light-emitting diode (Ocean Optics, Dunedin, FL, USA) with an intensity peak at 395 nm. Simultaneously, the emitted fluorescence generated by the fluorescent dye was transmitted to the spectrometer through another branch of the bifurcated optical fiber. The software OOIBase 32 (Ocean Optics, Dunedin, FL, USA) was used to automatically determine and analyze the fluorescence intensity.

2.3. In vitro experiments

The in vitro experiments were performed to characterize the pH sensor. The buffer solution consisted of 20 mmol/L 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) titrated to the desired pH, ranging from pH 6.0 to 8.0 in increments of 0.1 pH units, using 1 mol/L sodium hydroxide, with the pH value confirmed by a pH meter (Shanxin Cor., Shanghai, China). The pH sensor was immersed into the buffer solutions, and the fluorescence intensity peak at 510 nm was recorded as the pH sensor was excited at 395 nm. We examined the fluorescence properties and the accuracy of the pH sensor in a wide range of buffer solutions containing different concentrations of NaCl, KCl, $CaCl_2$, $MgSO_4$ or under different crystal, colloidal osmosis and temperature conditions ranging from 35 °C to 41 °C in a stepwise manner, similar to that previously described by Jiang et al. (2008). Gas, especially oxygen, is often an important factor of fluorescence quenching; therefore, the dried pH sensor was sequentially exposed to pure oxygen, CO_2 and nitrogen to examine the potential impact of oxygen and CO_2 on fluorescence intensity changes CO_2. To test the selectivity of the fluorosensor in different solutions, we immersed the sensor into different aliquots of sodium lactate Ringer's solutions that had each been titrated to a pH ranging from 6.0 to 8.0 with CO_2, diluted hydrochloric acid, sodium bicarbonate or lactic acid.

2.4. In vivo experiments

A total of 12 healthy, adult New Zealand white rabbits, 6 male and 6 female, weighing 2.5–3.5 kg, were purchased from the animal center of Fudan University. Animals were anesthetized with urethane (1 g/kg), injected through an ear vein and laid in a supine position. The trachea was intubated with a bifurcated glass tube, and then, the left femoral artery was exposed and cannulated with a 22-G catheter for blood gas analysis (Medica Easy Blood Gas analyzer, Medica, Bedford, MA, USA). The right carotid artery was isolated and cannulated using two 18-G catheters directed endocentrically and exocentrically to establish a collateral circulation system. The optic fiber pH sensor was inserted into the proximal catheter through its embranchment.

After placement of the sensor, the rabbits were stabilized for 30 min before conducting the experiment. To create acute metabolic acidosis or alkalosis, 5% sodium bicarbonate (3 ml/kg) was slowly injected through the ear vein every 30 min to gradually raise the blood pH to approximately 7.6, followed by 0.5 mmol/L hydrochloric acid (1.5 ml/kg) injected intraperitoneally every 30 min to slowly decrease the blood pH level to approximately 7.0. To create respiratory acidosis or alkalosis, six rabbits were paralyzed by pancuronium (1 mg/kg intraperitoneal) and mechanically ventilated with ambient air (tidal volume 8 ml/kg, respiratory rate 40 respirations/min) using an animal ventilator (Chengdu Apparatus Factory, Sichuan, China). Acute respiratory alkalosis was produced by gradually increasing the respiratory rate to 90 respirations/min in increments of 5 respirations/min. Acute respiratory acidosis was produced by gradually decreasing the respiratory rate to 15 respirations/min in decrements of 5 respirations/min (Jayaraman et al., 2001). Arterial blood samples (0.3 ml) were drawn anaerobically into 1 ml heparinized syringes at 5-min intervals and analyzed immediately using a BGA. Fluorescence intensities were recorded simultaneously on blood samples. The sensor was calibrated in vivo using the arterial blood gas samples. The rabbits were euthanized by injection of 50 ml of air into the ear vein at the end of each experiment.

2.5. Statistical analysis

The fluorescence intensity is presented as the mean ± SD. We tested the correlation between fluorescence intensity and pH measured by the pH meter or BGA. Linear regression and Bland–Altman analyses (Bland and Altman, 1986) were used to compare the pH values obtained by the sensor and the traditional BGA, with $P < 0.05$ considered significant. We reported bias ± SD, precision ± SD, and 95% limits of agreement, calculated as mean bias ± 1.96SD, where the bias is the mean difference of paired BGA fluorosensor measurements and the precision is the average of the absolute difference of paired BGA fluorosensor measurements.

3. Results and discussion

The probe was kept away from scattered ambient light during the experiments. The pH sensor exposed to HEPES buffer solutions or blood had strong, stable emission peaks at 518 nm when excited at 395 nm (Fig. 1A).

3.1. Application in vitro

3.1.1. Correlation between changes of fluorescence intensity and the pH of different buffer solutions

The fluorescence spectra of the pH sensor in HEPES buffer solutions of varying pH levels are shown in Fig. 1B. The fluorescence intensity decreased with increasing pH ranging from pH 6.0 to 8.0 (Fig. 1C). When the solution changed from pH 6 to pH 8, the fluorescence intensity decreased approximately 24% from 208.60 ± 1.25 to 158.73 ± 0.49 arbitrary units (a.u.), an average of 1.8% per 0.1 pH units, showing a negative linear relationship with pH ($n = 32$, $r^2 = -0.99$, $P < 0.0001$). The calibration curve of the pH sensor was constructed by plotting the measured fluorescence intensity values versus the recorded pH values. Based on these data, the following linear equation, pH = −0.0379 (intensity) + 14.034, was determined for the conversion of the fluorescence intensity into pH values.

3.1.2. Reproducibility, reversibility and response time

The reproducibility and reversibility of the sensor were evaluated by analyzing fluorescence intensity when exposed to buffer solutions at pH levels of 6, 6.35, 6.53, 6.8, 7.09, 7.18, 7.52 and 8 (Fig. 1D). The sensor responded immediately to the changes of pH in the solutions, suggesting that the response of the pH sensor should be fast enough to show real-time changes in blood pH levels. We