Blood gas analyzer utility in evaluating oxygen kinetics of the aqueous humor

Utilidade de um analisador sanguíneo de gás para a avaliação cinética do oxigênio no humor aquoso

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ABSTRACT

Purpose: To measure the partial pressure of oxygen (PO₂) and carbon dioxide (PCO₂) and the pH of aqueous humor (AH) and arterial blood samples from rabbits using a blood gas analyzer.

Methods: Twenty New Zealand rabbits were anesthetized intramuscularly with ketamine and xylazine and were then allowed to breathe room air. Using a gas blood analyzer, arterial blood and AH samples were analyzed for PO₂, PCO₂, and pH.

Results: The mean arterial blood pressure was 87.14 ± 15.0 mmHg. The mean blood and AH PO₂ were 95.18 ± 11.76 mmHg and 88.83 ± 9.92 mmHg, the mean blood and AH PCO₂ were 25.86 ± 5.46 mmHg and 29.50 ± 5.36 mmHg, and the mean blood and AH pH were 7.38 ± 0.06 and 7.33 ± 0.09, respectively.

Conclusions: The blood gas analyzer was easily employed to evaluate the aqueous humor in rabbits. When comparing the results of studies evaluating aqueous PO₂, care should be taken to determine the methods used in these studies.

Keywords: Aqueous humor; Blood gas analysis; Oxygen/physiology; Carbon dioxide/physiology; Rabbits

RESUMO

Objetivo: Medir a pressão parcial de oxigênio (PO₂) e dióxido de carbono (PCO₂), e o pH de humor aquoso (AH) e de amostras de sangue arterial de coelhos.

Método: Vinte coelhos New Zealand foram anestesiados por via intramuscular com cetamina e xilazina, em seguida, foram liberados a respirar o ar ambiente. Utilizando um analisador sanguíneo de gás, amostras de sangue arterial e AH foram analisadas para PO₂, PCO₂ e pH.

Resultados: A pressão arterial média foi de 87,14 ± 15,0 mmHg. A PO₂ do sangue e AH foi 95,18 ± 11,76 mmHg e 88,83 ± 9,92 mmHg, a PCO₂ do sangue e AH foi de 25,86 ± 5,46 mmHg e 29,50 ± 5,36 mmHg, o pH médio do sangue e AH foi 7,38 ± 0,06 e 7,33 ± 0,09, respectivamente.

Conclusões: O analisador de gás no sangue foi facilmente empregado para avaliar o humor aquoso em coelhos. Quando se compararam os resultados de estudos que avaliaram PO₂ do humor aquoso, deve ser tomado cuidado para determinar os métodos utilizados nestes estudos.

Descritores: Humor aquoso; Gasometria; Oxigênio/fisiologia; Dióxido de carbono/fisiologia; Coelhos

INTRODUCTION

The aqueous humor (AH) is critical to the maintenance of avascular eye structures, such as the cornea and the lens. It provides nutrients and removes the metabolites from these avascular structures. Oxygen is supplied to the AH via a transcorneal passage from the atmosphere, the limbal circulation, the arterioles of the ciliary body, and the iridial vessels.

Contact lenses, especially those with low oxygen permeability, dramatically hinder the passage of atmospheric oxygen to the cornea, causing the oxygen concentration in the corneal stroma and in the AH to decrease. This hypoxia may give rise to oxygen flux from the AH to the cornea, resulting in a decrease of AH oxygen tension.

A high incidence of eye surgery has been reported to be related to inadequate ventilation to the eye structures in patients under local or general anesthesia. As corneal endothelial cells are not counted before prescribing contact lens or before most intraocular surgeries, clinicians must maintain an adequate oxygen supply to the anterior chamber via the transcorneal passage and systemic circulation to avoid causing additional alterations in corneal endothelial pump function.

Most data regarding oxygen kinetics in the anterior chamber of the eye originate from indirect fluid measurements, including the application of polarographic electrode analysis inside the eye or on the corneal surface, orcal scanning fluorometry, or optical oxygen sensors and from direct measurements of the AH, using a blood-gas analyzer, obtained via anterior chamber paracentesis.

To the best of our knowledge, no data of simultaneous evaluations of the PO₂, PCO₂, and pH of AH and arterial blood samples exist. In the present study, we aimed to simultaneously measure the PO₂, PCO₂, and pH of blood and AH samples from rabbits.

METHODS

Adult male New Zealand rabbits, weighing between 2000 g and 3500 g, were obtained for this study and were treated during all experiments in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Ethics Committee of Canakkale Onsekiz Mart University School of
Medicine. The rabbits were evaluated for ocular and systemic problems, and only healthy rabbits were included in the study. Throughout the experimental period, the animals were fed with standard rabbit food and had access to water. The experiments were conducted between 09:00 and 16:00. All measurements were conducted at sea level. All the rabbits were made to fast overnight (approximately 8 h) before undergoing the experimental procedures.

On the day of the experiment, each rabbit was carried into the operating room. The left main auricular artery was catheterized for arterial blood sampling, and the rabbit was monitored by electrocardiography. The rabbit was anesthetized intramuscularly with 35 mg/kg ketamine (Ketasol, Richter Pharma AG) and 5 mg/kg xylazine (Rompun®, Bayer Healthcare LLC).

One milliliter of arterial blood was obtained from the rabbit’s ear artery; subsequently, using a heparinized tuberculin syringe with a 24-G needle, 0.2 ml AH was obtained from the right eye of the animal by anterior chamber paracentesis while it breathed room air. If an air bubble was visually observed in the syringe, the blood sample was discarded, and the rabbit was excluded from the study. All included samples were transferred immediately to the blood gas analyzer (Gasstat 600 Series Blood Gas Analyzer, Techno Medica Co. Ltd., Yokohama, Japan) that was actively running in the operating room and were analyzed for PO2, PCO2, and pH.

All data were analyzed using SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL, USA). Measurements were presented as mean ± standard deviation. The Shapiro-Wilk test was used to identify the normality of distribution. The Spearman rank correlation test was used to determine the relationship between measures of arterial and AH samples. A p value <0.05 was considered to be statistically significant.

RESULTS

Blood and AH samples in the present study were obtained from 20 rabbits under general anesthesia, and both samples were analyzed for PO2, PCO2, and pH. Mean arterial blood pressure was 85.7 ± 7.9 mmHg and 112.3 ± 6.2 mmHg, respectively (6). The arterial blood PO2 and PCO2 values were not correlated with pH (Spearman ρ=-0.068, p=0.777 and 0.212, respectively). The arterial blood pH was correlated with AH pH (Spearman ρ=0.457, p=0.043).

DISCUSSION

Corneal homeostasis depends to a great extent on the amount of energy obtained by the aerobic Krebbs cycle and the anaerobic Embden-Meyerhof pathway. In case of low oxygen levels during contact lens wear, the cornea shifts the anaerobic Embden-Meyerhof pathway, resulting in stromal lactic acid accumulation and an acidic shift in the stromal pH. The increase in stromal lactic acid creates an osmotic load and decreases the pumping function of the endothelium from prolonged corneal hypoxia; both of these changes affect the increase in stromal edema. Besides corneal stromal changes, endothelial blebs, endothelial polymegatism, and pleomorphism are also potentially related to contact lens wear.

Because of the dynamic interaction between the cornea and AH, its biochemical analysis has long received much attention. It must also be stressed that the biochemical analysis of aqueous fluid has not been validated using different types of analyzers; as such, AH analysis has no ‘gold standard.’ In fact, there is poor agreement regarding AH oxygen tension in reports using different analytical methods (24-4,17). Using polarographic oxygen electrodes, Stefansson et al. (16) reported a mean aqueous PO2 of 23 ± 2 mmHg in rabbits, whereas Fitch et al. (10) reported a mean aqueous PO2 of 63 ± 9 mmHg in rats breathing room air. Using ocular scanning fluorometry, mean aqueous PO2 in rabbits was 23 ± 3 mmHg (range, 20-29 mmHg) (15). Shui et al. (16) used an optical oxygen sensor (optode) in the center of the anterior chamber, reported an aqueous PO2 of 27 ± 2 mmHg in rabbits breathing 20% oxygen and >100 mmHg when the animals were ventilated with 60% oxygen; they did not measure oxygen levels over 100 mmHg, as the sensor could not reliably measure higher oxygen values.

Jampol et al. reported mean aqueous PO2 levels in rabbits kept under normobaric conditions breathing room air as 63.5 ± 12.3 mmHg using a blood gas analyzer (17). Sharifipour et al. used a blood gas analyzer in human patients breathing 21% oxygen undergoing cataract surgery under local anesthesia, and reported blood and AH PO2 at 85.7 ± 7.9 mmHg and 112.3 ± 6.2 mmHg, respectively (17).

The key aspect of polarographic oxygen electrodes and optodes is their ability to measure PO2 in different regions of the eye. In reports using these electrodes, lower PO2 measurements may be attributed to the oxygen consumption property of the electrodes (16,20).

In the present study, the mean aqueous PO2 levels were in partial accord with studies using polarographic oxygen electrodes in rabbits and rats (18) and with studies using blood gas analyzers in rabbits and humans (6); however, PO2 levels in this study were higher than those in studies using polarographic oxygen electrodes in rabbits and rats (18). PO2 levels were also higher in the present study than in studies using ocular scanning fluorometry (15) and optodes (16) in rabbits.

The differences between the studies may have been due to air contamination during paracentesis when using a blood gas analyzer. To avoid this complicating factor, we discarded samples if air contamination occurred. A second possible explanation for the higher results in studies using a blood gas analyzer may be delayed analysis. Using plastic syringes, which do not have a good oxygen barrier, may lead to overestimated PO2 levels in aqueous samples. To avoid this complicating factor, all samples in the present study were analyzed by a blood gas analyzer, which was ready for use and in the operating room, within a minute of taking the sample.

The higher values reported in some earlier studies compared with those reported in the present study may be related to the very large corneal surface of the subject’s eye in relation to eye volume. With regard to the Shui et al. study, in which the breathing conditions of rabbits had been increased from 20% to 60% oxygen, using an optode reportedly caused the PO2 to increase by factors of approximately 5 to over 12 times in different parts of the eye. Disparities in AH PO2 levels between the different studies may be the result of the lack of standardized ventilation during sampling. To prevent these inconsistencies, the blood and AH samples must be analyzed simultaneously and arterial pressures must be measured. In addition, the altitude at which the study was conducted can affect the partial pressure of the inspired oxygen, and may contribute to these differences. The present study was conducted at sea level.

The intent of the present study was to measure the mean PO2, PCO2, and pH values of the AH simultaneously with those of the blood; due to the limitations of the blood gas analyzer, the evaluation of O2 and CO2 kinetics were beyond the scope of the study. The significance of these AH measurements lies in the importance of oxidative (e.g., cataract and glaucoma) and hypoxic (e.g., rubiosis iridis) events that are a characteristic of numerous anterior segment pathologies. Measuring AH PO2, PCO2, and pH levels with an

Table 1. Arterial blood and aqueous humor gas measurements in rabbits

<table>
<thead>
<tr>
<th>Samples</th>
<th>PO (mmHg) mean ± SD</th>
<th>PCO (mmHg) mean ± SD</th>
<th>pH mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood samples</td>
<td>95.18 ± 11.76</td>
<td>25.86 ± 5.46</td>
<td>7.38 ± 0.06</td>
</tr>
<tr>
<td>Aqueous humor samples</td>
<td>88.83 ± 9.92</td>
<td>29.50 ± 5.36</td>
<td>7.33 ± 0.09</td>
</tr>
</tbody>
</table>
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An easily accessible single device, i.e., the blood gas analyzer, gives investigators an opportunity to examine anterior segment pathologies. In conclusion, when comparing the results of studies measuring PO$_2$ of the AH with the same or different devices, respiratory and hemodynamic parameters, which affect blood and tissue oxygenation during the study, must be taken into account. The present study simultaneously measured the PO$_2$, PCO$_2$, and pH of the AH as well as that of arterial blood samples in rabbits under ketamine/xylazine anesthesia. The use of the blood gas analyzer in the operating room ensured that the study rapidly and easily measured the PO$_2$, PCO$_2$, and pH of the AH.

REFERENCES