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EFFECT OF HYPOVENTILATION ON BLOOD IONS AND GASES ON EXPERIMENTAL RAT MODEL

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Abstract

Hypoventilation is the inadequate breathing that is inadequate to meet the needs of the body or reduced lung function. The objective of this study was to investigate the effect of hypoventilation on blood ions and gases in the rat specially focusing on pH and blood magnesium (Mg^{2+}); ionized and total, in the subject under experimental hypoventilation. Male Sprague-Dawley rats (250-300g) was selected and mechanical hypoventilation was applied by decreasing the rate of ventilation to as low as 40 cycles/min and the stroke volume was decreased down to 1.2 ml and the condition was continued during 20 minutes. The blood ions and gas was measured in the same experimental subject before and after hypoventilation. NOVA analyzer using an ion selective sensor for measuring ionized Mg^{2+} (iMg^{2+}), calcium (Ca^{2+}), potassium (K^{+}), chlorine (Cl^{-}), pH, Hematocrit (Hct) and blood gases. Total Mg^{2+} (tMg^{2+}) in blood, plasma and RBC was measured by atomic absorption spectrophotometer. By direct comparison of initial values and experimental data in whole blood and red cells, the model has been shown to give realistic estimations of iMg^{2+} and tMg^{2+} in response to changes in each of these parameters. The blood pH significantly ($p<0.001$) decreased in the hypoventilated group and pCO_2 significantly increased ($p<0.001$) compared to the normal. In RBC, iMg^{2+} varies with the tMg^{2+}, the concentration and availability of metabolites that bind Mg^{2+}, the pH and the state of oxygenation. Only the significance difference was noticed in case of K^{+} ($p<0.05$), iCa^{2+} ($p<0.05$) and iMg^{2+} ($p<0.001$) concentration. Hypoventilation is major cause of respiratory acidosis which disrupts the ionic and gaseous homeostasis. The model will be a useful tool for further investigation of hypoventilation and may reveals scopes for treatment and management strategy to recover the clinical condition.

Keywords: Hypoventilation, blood gases, blood ions, blood magnesium, rat model.

Introduction

Magnesium serves as a cofactor for more than 300 cellular enzymes, predominantly related to energy metabolism (Fleet and Cashman, 2001; Shils, 1999). Abnormalities of serum Mg^{2+} may be most underdiagnosed serum electrolyte disturbance in clinical practice with an incidence ranging from 12.5% to 20% in routine determination (Whang, 1987), and despite its importance, routine serum Mg^{2+} determination has been proposed to be an unrecognized need (Whang *et al.,* 1990). Therapeutic condition with Mg^{2+} in excess causes hypoventilation but the comprehensive study on blood Mg^{2+} in subject under hypoventilation condition is still unavailable. Extracellular Mg^{2+} is bound to serum albumin and plasma Mg^{2+} levels do not reliably reflect total body Mg^{2+} stores. Mg^{2+} is necessary for the movement of

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sodium, potassium, and calcium into and out of cells, and Mg^{2+} plays an important role in stabilizing excitable membranes (Grubbs and Maguire, 1987; Romani and Scarpa, 1992). Low potassium in combination with low Mg^{2+} is a risk factor for severe arrhythmias. Thus, Mg^{2+} balance is closely tied to sodium, calcium, and potassium balance (Nishimuta *et al.,* 2006). However, despite the importance of Mg^{2+} in nutrition and health, there are no adequate, sensitive and specific markers to assess Mg^{2+} status in animals. Mg^{2+} status is currently evaluated using various biochemical markers like total and ionized plasma Mg^{2+}, red blood cell (RBC) Mg^{2+}, however, they all present some limitations. In cellular respiration or metabolism gases cross the respiratory surface by diffusion, many also have a mechanism to maximize the diffusion gradient by replenishing the source and/or sink. Control of respiration is due to rhythmical breathing generated by the phrenic nerve to stimulate contraction and relaxation of the diaphragm during inspiration and expiration. Several studies have reported the difficulty in detecting hypoventilation in patients with undergo sedation for GI, dental, and other endoscope procedures and failure to diagnose severe hypoventilation in the preoperative period (Maeda *et al.,* 1992). The early postoperative period may be associated with hypoventilation caused by respiratory depression and inability to maintain an adequate airway (Parr *et al.* 1991, Bonnardot *et al.,* 1978). Symptomatic hypermagnesemia usually requires both increased intake of the ion and abnormal renal function. Therapy included ventilatory support (Fassler *et al.,* 1985), intravenous calcium, and fluids, and recovery was complete. Extremely high serum Mg^{2+} levels may produce a depressed level of consciousness, bradycardia, cardiac arrhythmias, hypoventilation (Higham *et al.,* 1993), and cardiorespiratory arrest. Though, there is much more evidence is available that ventilation therapy is frequently practiced in case of hypermagnesemia or Mg^{2+} toxicity cases, but the blood Mg^{2+} level in case of hypoventilation is never been concerned. This study evaluated the blood Mg^{2+} concentration in subject under experimental hypoventilation in rat model. This study also investigated the effects of hypoventilation on blood ionized Mg^{2+}, Ca^{2+} and total Mg^{2+}, Ca^{2+} as well as several electrolytes and attempted to confirm the results describing a pattern of changes in blood gases, pH, and hematocrit.

Materials and Methods

Animal preparation

A number of 15 male Sprague-Dawley rats (250-300g) were purchased from the Bio-safety Research Institute of Chonbuk National University and were reared in an air-conditioned room of (25.0±1.0°C) temperature, 60-70% relative humidity and a day night light cycle were maintained. The rats were allowed free access to a standard rat feed and water.

Mechanical hypoventilation

The rat was anesthetized by Tiletamin (80 mg/kg i.p. with additional doses of 5 mg/kg i.p. as required). The initial ventilation rate was 80 cycles/min and the tidal volume of ventilation was ranged from 2.0 to 2.5 ml depending on body weight of the rat. Mechanical hypoventilation was applied by decreasing the rate of ventilation to as low as 40 cycles/min and the stroke volume was decreased down to 1.2 ml and the condition was continued during 20 minutes.

Measurement of gases, ions and total magnesium

The right carotid artery was cannulated for collection of arterial blood. The arterial catheters were flushed with heparinized saline. Ionized magnesium concentration as well as other electrolyte was measured in the freshly collected blood. Blood gasses were also measured at the same time using the ion-selective electrode. Nova stat profile was used to measure all the ions and gasses. Freshly drawn heparinized blood (6 IU. of sodium heparin pro 1 ml of blood) was centrifuged (15 min, 3000 rpm). The serum was separated from the erythrocytes and the coating of lymphocytes was removed. Ionized Mg^{2+} concentration in serum was also measured in fresh samples by above method. Serum tMg^{2+} concentrations were determined by flame atomic absorption spectrometry using Analab 9200, Seoul, Korea. with a Mg^{2+} hollow cathode lamp (Hunt, 1969) and the instrumental settings of the spectrophotometer were; wavelength 285.2 nm; slit width 2 nm; power supply 3 mA; acetylene flow 2.8 L/min; air flow 7 L/min; aspiration rate 6 ml/min; burner height 7; and time of measurement for every sample 5 second. To describe the method briefly, serum samples were diluted 1:30 with 0.5% (*w*/*v*) lanthanum chloride in 0.12 N hydrochloric acid. Serum tMg^{2+} concentration was determined from a linear standard curve established by 0-0.8 mg/L (ppm) atomic absorption standard for Mg^{2+} (SCP Science, Canada). Aliquot of erythrocytes were washed three times with saline solution (9 g/L), the buffy coat was removed and erythrocytes were hemolyzed in distilled water (1:10). Total Mg^{2+} content in erythrocytes was also measured by above method.

Statistical analysis

The results are presented as a means ± standard error of the mean (SEM). The data were analyzed using the Student's t-test and the repeated-measures analyses of variance ANOVA followed by the Bonferroni test. A probability of less than 0.05 was taken as a statistically significant difference.

Results

Effects of hypoventilation on pH and blood gas

After 20 minutes of hypoventilation (Decreased tidal volume and rate of respiration mechanically using ventilator), the hematological status of pH, PO_2, O_2ct, O_2sat, and AO_2 were measured and we found significant decrease of all parameters in comparison with that of control. On the other hand blood glucose and PCO_2 were found significant increase, and there is no change of hematocrit and hemoglobin were found.

Table 1 shows the pH of the blood and partial pressure of blood gas; it also shows the glucose, Hct, and Hb in the freshly collected blood. No abnormalities were observed on gross examination at autopsy, and no animals were excluded from data analyses. Tweenty minutes of hypoventilation, the pH decreased significantly *($p<0.001$)*, which causes moderate respiratory acidosis in accordance with control. When the frequency of ventilation was decreased under physiological conditions in anesthetized animals, pCO_2 increased from a baseline value of 46.1 ± 2.8 to 64.4 ± 5.5. The increase of blood pCO_2 is 39.70% and this difference was statistically significant ($p<0.01$). When the frequency of ventilation was returned to baseline values, tissue pCO_2 values were restored to approximately

Table 1. Effects of hypoventilation on pH, blood gas and hematological parameters

Parameters	Control	Hypoventilation	(%) Increase (↑) or decrease (↓)
pH	7.438 ± 0.013	7.285± 0.014***	2.06 ↓
pCO_2 (Partial carbon dioxide tension)	46.1 ± 2.8	64.4 ± 5.5**	39.70 ↑
pO_2 (Partial oxygen tension)	85.7 ± 7.2	68.1 ± 3.3*	20.54 ↓
O_2ct (Oxygen content)	19.0 ± 0.4	17.6 ± 0.5*	7.37 ↓
O_2sat (Oxygen saturation)	94.8 ± 1.3	87.9 ± 1.8**	7.28 ↓
AO_2 (Alveolar oxygen)	94.1 ± 3.4	72.3 ± 6.8*	23.17 ↓
Glucose	237 ± 18	321± 29*	35.44 ↑
Hct (Hematocrit)	42 ± 1	40 ± 1	4.76 ↓
Hb (Hemoglobin)	13.6 ± 0.4	12.4 ± 0.6	8.82 ↓

Results are expressed as mean ± SE and ****P<0.001, **P<0.01, *P<0.05* is taken as significant difference between hypoventilation group vs control group, (n=15)

baseline values. On the other hand, pO_2, O_2ct, O_2sat and AO_2 were decreased significantly. A significant increase of blood glucose was found (*p<0.05*). This may be due to lack of sufficient oxygen required for aerobic metabolism after prolong hypoventilation. There were little hemodynamic changes or differences in the Hct and Hb gradients. Arterial blood gas analysis is widely available in hospitals and the direct measurements of pH, pO_2 and pCO_2 are the most precise in medicine. After a certain period of time, pO_2 changes in the blood with acute hypoxia which proves that hypoventilation decreases pH of blood which causes respiratory acidosis.

Effect of hypoventilation on blood ions

Plasma ion concentrations during hypoventilation and in normal state are summarized in Table 2. After 20 minutes of mechanical hypoventilation, the changes of the blood ions are expressed as (%) increase or decrease in comparison with that of control. The ionized and pH-normalized ionized Mg^{2+} concentrations, ionized and pH-normalized ionized Ca^{2+} concentrations, Na^+, K^+ and Cl^- concentrations were measured in control and hypoventilated group. The blood ionized magnesium increased 21.15% (*p<0.001*), normalized Mg^{2+} ion to pH 7.4 increased 15.09% (*p<0.001*), and Ca^{2+} ion levels significantly increased 4.88% (*p<0.05*), but normalized ion Ca^{2+} to pH 7.4 decreased 20%, which wat not significant in comparison with that of control. The ratio of iCa^{2+}/iMg^{2+} is significantly (*p<0.001*) decreased (11.86%) and also the ratio of nCa^{2+}/nMg^{2+} was found decreased (15.19%) significantly (*p<0.001*), K^+ ion was found a little increase (4.76%) but this increase was not significant. Plasma Na^+ and Cl^- were unaffected by hypoventilation. Plasma K^+ ions increased significantly in hypoventilated group and differed between the groups which caused 4.76% increment (*p<0.05*) in comparison with control group.

Table 2. Effect of hypoventilation one the blood ions concentration in compared with the control group

Items	Control	Hypoventilation	(%) Increase (↑) or decrease (↓)
Na^+	138 ± 1	137 ± 1	0.72 ↓
K^+	4.2 ± 0.2	4.4 ± 0.1*	4.76 ↑
Cl^-	100 ± 1	100 ± 1	
iCa^{2+}	1.23 ± 0.02	1.29 ± 0.02*	4.88 ↑
iMg^{2+}	0.52 ± 0.01	0.63 ± 0.02***	21.15 ↑
iCa^{2+}/iMg^{2+}	2.36 ± 0.04	2.08 ± 0.05***	11.86 ↓
nCa^{2+}	1.25 ± 0.02	1.21 ± 0.02	3.20 ↓
nMg^{2+}	0.53 ± 0.01	0.61 ± 0.01***	15.09 ↑
nCa^{2+}/nMg^{2+}	2.37 ± 0.05	2.01 ± 0.06***	15.19 ↓

Results are expressed as mean ± SE and ***$P<0.001$, **$P<0.01$, *$P<0.05$ is taken as significant, hypoventilation group v/s control group, n=15. (nCa^{2+} and nMg^{2+} are normalized Ca^{2+} and Mg^{2+} respectively to pH 7.4)

Effect of hypoventilation on total magnesium in blood plasma and RBC

Total magnesium in whole blood, plasma, and RBC were measured atomic absorption spectrophotometrically. In control subjects the mean tMg^{2+} was found as follows: whole blood tMg^{2+}1.248 ± 0.059 mmol/L, plasma tMg^{2+} 0.860 ± 0.035 mmol/L, and RBC tMg^{2+} 0.67-0.96 mmol/L. The whole blood and plasma tMg^{2+} significantly ($p<0.01$) increased and RBC tMg^{2+} was found significantly ($p<0.01$) decreased (Fig. 1). The rat under hypoventilation, the mean whole blood tMg^{2+} 1.434 ± 0.048 mmol/L, plasma tMg^{2+} 1.258 ± 0.090 mmol/L, and RBC tMg^{2+} 1.524 ± 0.057 mmol/L were found. This data reveals an increase of blood tMg^{2+} 14.90% ($p<0.01$) and plasma tMg^{2+} 46.27% ($p<0.001$) and decrease of RBC tMg^{2+} 15.66% ($p<0.01$). Although less than 1% of the total body magnesium is present in blood, the determination of this parameter is mainly done for blood serum or plasma in routine clinical analysis. The whole blood and plasma tMg^{2+} concentrations were significantly higher and erythrocyte tMg^{2+} concentration was significantly lower in hypoventilation rats than in controls.

Effect of hypoventilation on blood ionized Mg^{2+} and ionized Ca^{2+}

Ionized and pH-normalized ionized magnesium and concentrations, sodium and potassium concentrations, and Hct were measured prior to treatment. Plasma iCa^{2+} increased significantly ($p<0.05$) relative to controls after 20 minutes causes 4.76% increment. Plasma iMg^{2+} increased 21.15% and was significantly ($p<0.001$) different. Hypoventilation increased plasma Ca^{2+}, Mg^{2+}, and K^+ and had no effect on plasma Na^+ and Cl^-. The only laboratory value strongly correlated with iMg^{2+} concentration was pH-normalized iMg^{2+} concentration. In the control group iMg^{2+} concentrations differed by the effect of hypoventilation. Hypoventilated rat showed higher iMg^{2+} concentrations compared with those of normal control.

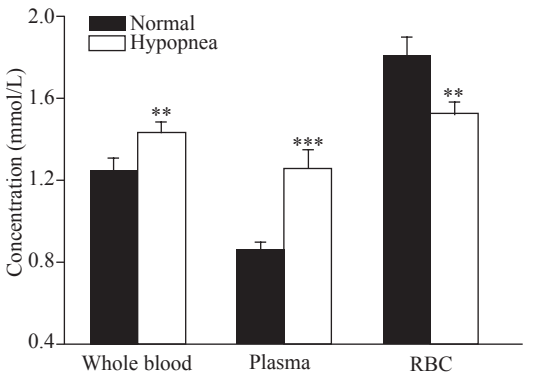

Fig. 1. Whole blood, Plasma and RBC total Mg^{2+} concentration in controls (normal) and hypoventilated group of rats, Values are means ± S.E.M., n=15 per treatment for each ion and **p<0.05, **p<0.01, ***p<0.001,* hypoventilated group vs normal rat.

Discussion

Ventilation can be defined as the movement of a volume of air in to and out of the lungs, removing carbon dioxide from the blood and providing oxygen. The purpose of determining oxygen content of the blood is to oxygen delivery to cells. Increase of partial carbon dioxide tension and decrease of partial oxygen tension, oxygen content, oxygen saturation, alveolar oxygen confirm that it is caused by hypoxic state. Calcium and magnesium have many important physiologic functions, and measurement of iCa^{2+} and iMg^{2+} concentrations in plasma provides useful information for clinical diagnosis and management. Therefore, several methods of the measurement of total cellular Mg^{2+} also in erythrocytes tMg^{2+} have been described. However, the concentration of intracellular iMg^{2+} is much more physiologically relevant and therefore of special interest. The knowledge of a relation between the ionized and the total magnesium concentration in serum and erythrocytes together with the knowledge of exchange of erythrocyte-serum gives a comprehensive biochemical transformations occurring in the organism. Hypoventilation can be due to respiratory disease, acute airway obstruction; parenchymal lung disease, chronic obstructive diseases, etc. and leads to a reduced blood Mg^{2+} level (Lopez *et al.,* 2002). But the present study found increased blood Mg^{2+} level in experimental hypoventilation might be due to sudden acute hypoventilation.

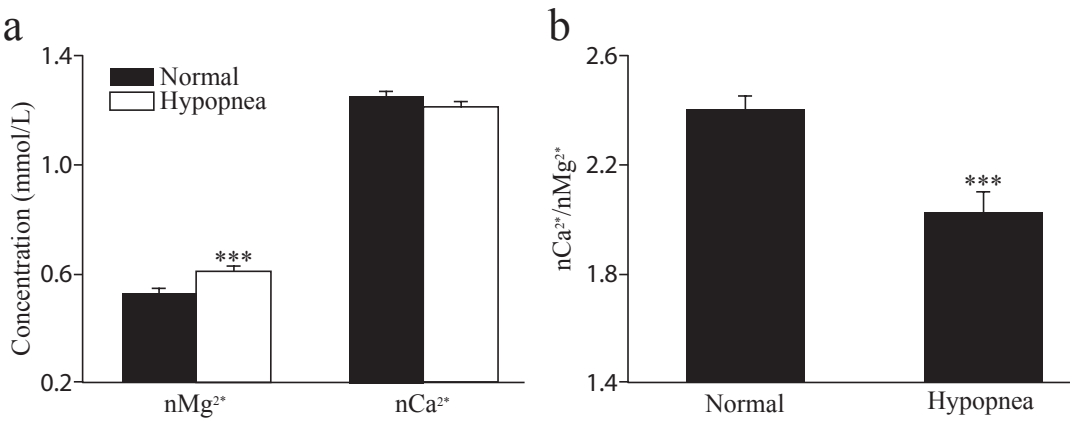

Fig. 2(a). Effect of hypoventilation on blood iMg^{2+} and iCa^{2+}, (b) Ratio of normalized calcium and normalized magnesium. Values are means ± S.E.M., n=15 per treatment for each ion and **p<0.05, **p<0.01, ***p<0.001,* hypoventilated group vs control group/normal rat.

Pulmonary hypoventilation and the state of pH and arterial blood gases

The parameters pH for acid or base, HCO_3^-, pO_2, SO_2 for oxygenation status, pCO_2 for ventilation status are best concerned during hypoventilation. All these parameters have specific and important impact on respiratory disorders. Oxygen content reflect the amounts of O_2 dissolved in plasma, which is related to total O_2 carried in the blood that provides arterial oxygenation. Respiratory acidosis and the drop in pH is explained by the change in pCO_2. In the primary respiratory acid base disturbances, acute respiratory acidosis is the consequences of acute hypoventilatory failure with prolong CO_2 retention, which is commonly found in chronic obstructive lung disease and renal compensation in sets of mitigation of academia (Flatman and Membr, 1984). In essence, hypoventilation occurs when the respiratory system fails to meet the demand for ventilatory work either the ability to supply ventilatory work has become limited, such as in the case of decreased respiratory drive or respiratory muscle weakness, or when the demand for ventilatory work becomes excessive, such as in the case of severe airway obstruction (Shahrizaila *et al.,* 2006). The carbon dioxide level rises leading to inadequate oxygen in the blood. Gas exchange or respiration takes place at a respiratory surface– a boundary between the external environment and the interior of the body. Acute respiratory acidosis is characterized by low pH and high pCO_2. If the condition persists, bicarbonate excretion in the kidneys will decrease and acidosis will be partly or totally compensated for by increased bicarbonate concentration in the blood. Compensated respiratory acidosis is characterized by only slightly low pH, high pCO_2 and high bicarbonate concentration (Truchot, 1981). Thus, pH may be seen as composed of a respiratory component and a metabolic component. Any primary disturbance of the acid-base equilibrium is met by a secondary physiological attempt at compensation to normalize pH. In this respect, variations in alveolar ventilation and variations in renal acid-base excretion act as mutually opposing regulators. Alveolar hypoventilation with resultant respiratory acidosis is sought compensated by increased pCO_2 retention in the bloods (Table 1). A small change in pH represents large changes in actual H^+. A structured approach to the interpretation of arterial blood gases helps ensure that nothing is missed. Two basic steps should be followed. Firstly, pCO_2 and pH should be assessed. In essence this is the ventilation state (respiratory acid-base balance) and will automatically lead to an assessment of the metabolic acid-base balance. Secondly, arterial oxygenation should be assessed.

Relationship between acute hypoxia and blood ions

The role of Mg^{2+} is primarily a cofactor in intracellular biochemical reactions, and almost 99% of the total body Mg^{2+} can be found intracellular (Fawcett *et al.,* 1999). Approximately 40% of Ca^{2+} and 30% of Mg^{2+} are bound to proteins in human plasma. Changes of pH in the specimen affect the binding of these ions to plasma proteins, mainly albumin, because hydrogen ions compete with iCa^{2+} and iMg^{2+} for protein binding sites. Elin (2010); Romani (2007) studied the effect of pH on ionized Mg^{2+} test results by analyzing anaerobic serum samples from patients and reanalyzing those samples after

pH was increased by *in vitro* loss of carbon dioxide. Their findings differed according to the analytic system used: the NOVA CRT system (NOVA Biomedical, Waltham, Mass) showed a decreased iMg^{2+} concentration with increased pH, whereas the AVL system (9884 Electrolyte Analyzer, AVL Roche Diagnostics, Roswell, Ga) indicated that iMg^{2+} concentration was not affected by an increase in serum pH of up to even 0.6 units from the baseline value (7.37-7.52). Several studies also stated the influence of pH on iMg^{2+} with the microlyte Mg^{2+} system (Kone Instrument, Finland) in serum, and showed a constant exponential factor x in the Siggard-Anderson equation in predicting iMg^{2+} concentration at pH 7.40 (Julia *et al.,* 1999; Nishimuta *et al.,* 2006; Maeda *et al.,* 1992). This study has the similar changes of iMg^{2+} concentration in subject under hypoventilation in serum or whole blood to establish the relationship between iMg^{2+} concentration and pH in routine specimens encountered in clinical laboratories. Measuring serum tMg^{2+} is a feasible and affordable way to monitor changes in Mg^{2+} status. It involved in carbohydrate, lipid, protein and DNA metabolism, interacting either with the substrate or the enzyme directly (Romani, 2007). Magnesium is abundant within all cells and essential for animal life, but it has been forgotten and its general roles in cellular function are poorly understood. Other ions like Na^+, K^+ and Cl^- ion remain unchanged in comparison with control which indicate that acute hypoxia do not affect on those ions. During tissue hypoxia or anoxia degradation of cellular ATP causes magnesium free from ATP-Mg^{2+} binding form. Intracellular magnesium come to extracellular and increase blood magnesium level (Rahman *et al.,* 2009).

The data from the present study showing increased glucose concentration in blood is similar to the investigations by Pandurengan and Thorpe (2003). They found an increased level of blood glucose unto 9.6 mmol/L in case of hypoventilation. This might be due to the hypoxia or lack of oxygen induced by hypoventilation because tissue needs oxygen for glucose metabolism, if there is hypoxia, there may be anaerobic fermentation on the other hand increases level of glucose in blood. The change in hematocrit and hemoglobin is not significant except little variation.

Conclusion

In red cells, iMg^{2+} varies with the tMg^{2+}, the concentration and availability of metabolites that bind Mg^{2+}, the pH and the state of oxygenation. By direct comparison of normal values and experimental data obtained in whole red cells, the model has been shown to give realistic estimations of iMg^{2+} and tMg^{2+} in response to ventilation status along with other parameters. The model will be a useful tool for the estimation of iMg^{2+} in experiments designed to determine the effects of iMg^{2+} on metabolic and transport processes in red cells and may also be valuable for the intracellular calibration required for reliable iMg^{2+} measurement using atomic absorption spectrometry, microelectrodes or NMR techniques.

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