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On-line microdialysis sampling coupled with flame atomic absorption spectrometry for continuous in vivo monitoring of diffusible magnesium in the blood of living animals

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Abstract

A novel on-line microdialysis sampling coupled with flame atomic absorption spectrometry (FAAS) with an attractive application is reported. Microdialysates perfused through implanted microdialysis probes were directly introduced into the flame atomizer of a FAAS system using 0.2% HNO_3 as carrier solution at a nebulizer uptake flow rate of 6 ml min^{-1}. The interval for each determination was 90 s (60 s sampling time, 10 s read time and 20 s washing time). The analytical characteristics of the on-line microdialysis–FAAS system were validated as follows: linearity range, 0–300 mg l^{-1}; detection limit (3σ, $n = 7$), 0.53 mg l^{-1}; precision (R.S.D., $n = 50$), 4.1%. By comparing Mg levels in the blood of living rabbits with the results obtained from in vivo no net flux (NNF) method, the accuracy of the proposed on-line method was found to be good. The present method can be successfully applied to the in vivo monitoring of diffusible Mg in the blood of living rabbits after magnesium sulfate ($MgSO_4$) administration with a temporal resolution of 1.5 min.

Keywords: Microdialysis sampling; Flame atomic absorption spectrometry; Magnesium; In vivo

1. Introduction

Magnesium (Mg), the second most abundant intracellular cation and the fourth most abundant cation in human body [1], plays a key role as a cofactor in many essential enzymatic reactions that are pivotal in the metabolism of carbohydrate, lipid and proteins [2]. Previous studies frequently used total serum Mg concentration to estimate diseases associated with hypomagnesemia or hypermagnesemia. However, several of those studies [3–5] reported that total serum Mg concentration may not be an accurate reflection of body Mg stores. In the blood, Mg exists in protein-bound, complex-bound, and free ionized forms, and only the ionized Mg^{2+} is physiologically active [6,7]. Methods that specially apply to measure ionized Mg, using ion-selective electrodes for Mg^{2+}, are now available for routine determination of ionized Mg^{2+} concentration in clinical laboratories. However, existing ion-selective electrodes have suffered from a lack of sensitivity and relatively long response times and may not be available for continuous in vivo monitoring of ionized Mg^{2+} in living organisms.

Microdialysis [8–12], a powerful in vivo sampling technique used to obtain protein-free samples, allows monitoring within physiological environment with a minimum of disturbance to the animal. A microdialysis system has the characteristics that is easy to automate and can be on-line coupled with many analytical techniques such as liquid chromatography

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[13], capillary electrophoresis [14], mass spectrometry [15], flow-injection analysis [16], and electrochemical detection [17,18]. Recently, monitoring of diffusible Mg using in vivo microdialysis sampling techniques has been reported [19,20]. These studies used off-line collection of the microdialysis sample and subsequent analysis by either flame atomic absorption spectrometry (FAAS) [19] or electrothermal atomic absorption spectrometry (ETAAS) [20]. On-line in vivo microdialysis sampling coupled to suitable detection system may offer advantage over conventional methods of studying blood electrolytes, especially for magnesium which is easily affected by exposure to air and the change of pH [21]. Furthermore, on-line analysis could also avoid time-consuming and tedious sample pretreatment works which are encountered in most off-line analysis.

In our previous work, a hyphenated technique of on-line microdialysis sampling coupled to ETAAS has been developed for the determination of diffusible manganese in the brains of living rats [22]. However, the on-line microdialysis–ETAAS system is not suitable for the determination of diffusible Mg in blood because of the high Mg levels in blood. The reference value of Mg in blood is approximately 17.0–24.3 mg l^{-1} [5]. Even though the detection range of ETAAS for Mg analysis is lower than 0.001 mg l^{-1}, when the determination of diffusible Mg is performed using microdialysis coupled on-line with ETAAS, readings may fluctuate greatly due to on-line sample dilution. To overcome this drawback, it is possible to couple FAAS, the conventional analytical method for the determination of serum electrolytes, directly to microdialysis sampling system. However, a problem with many atomic spectroscopy instruments except for ETAAS is the large amount of sample volume needed for collection. Taking the advantage of the flow-through nature of FAAS, an on-line microdialysis–FAAS analytical system was proposed.

Microdialysis sampling coupled on-line with FAAS may provide advantages, such as in situ sampling and nearly real-time measurement, simplified sample preparation, rapid analyses, and the dynamic monitoring of diffusible electrolytes in living systems. In this study, we therefore devoted to develop a method for continuous in vivo monitoring of blood diffusible Mg using microdialysis sampling coupled on-line with FAAS. The optimal operating conditions and validated analytical performance were investigated by the proposed on-line microdialysis–FAAS system. The proposed method was applied successfully to determine the nearly real-time concentration of diffusible Mg in the blood of anesthetized rabbits after magnesium sulfate ($MgSO_4$) had been administrated.

2. Experimental

2.1. Reagents and vessels

High-purity water (18.3 MΩ cm) was prepared with a deionized water system (Milli-Q, Millipore) and used throughout the work. All of the reagents used were of the highest available purity and were at least of analytical grade. The perfusion solution was prepared by dissolving 0.9 g of NaCl (Merck, ultrapure grade) in 100 ml of high-purity water and the pH was adjusted to 7.2. The carrier solution was prepared by diluting 2 ml of HNO_3 (Merck, ultrapure grade) in 1000 ml of high-purity water. Stock solutions (1000 mg l^{-1}) of Mg were purchased from Merk. The working aqueous Mg standard solutions were diluted fresh daily with 0.9% NaCl.

Before each use, all containers and pipette tips were scrubbed in 20% nitric acid (Riedel-de Haën, Germany) overnight; then cleaned with high-purity water five times. The tubings used to connect all apparatuses were perfused with high-purity water to flush out contamination. In order to avoid contamination resulted from magnesium, metal-free syringes (10 ml Luer Tip syringe, mode 81601, Hamilton, USA) were used as the perfusion syringes throughout this work. Twenty millimeters polyetheretherketone (PEEK) tubing (0.25 mm i.d.) was fitted to the syringe, making a homemade metal-free needle.

2.2. Instrumentation

A schematic diagram of the on-line microdialysis–FAAS system for the continuous determination of diffusible Mg in the blood of living rabbits is shown in Fig. 1. Microdialysates perfused through a microdialysis probe were collected in a sample loop on a six-port on-line injection valve for direct injection into a flame atomizer by a nebulizer uptake flow.

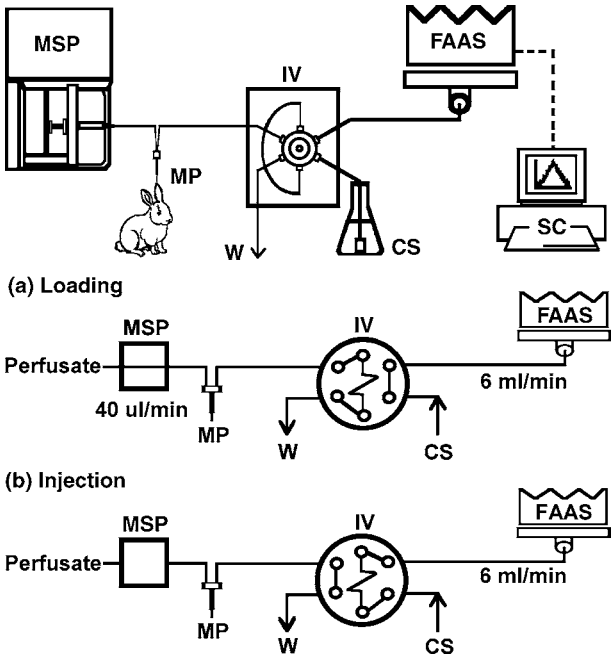

Fig. 1. Schematic diagram of the on-line microdialysis–FAAS system for continuous determination of diffusible Mg in the blood of living rabbits. MSP, microinjection syringe pump; MP, microdialysis probe; IV, injection valve; W, waste; CS, carrier solution; FAAS, flame atomic absorption spectrometer; SC, spectrometer computer.

The microdialysis system was purchased from Carnegie Medicine Associates (CMA, Stockholm, Sweden). The microdialysis sampling system consists of a microinjection syringe pump (CMA/100) and a 24 mm long microdialysis probe (CMA/20) with a 10 mm long and a 0.5 mm diameter polycarbonate membrane, which is metal-free and has a molecular mass cut-off of 20 kDa. Fluorinated ethylene polypropylene (FEP) tubing (50 cm long × 0.12 mm i.d.); (internal volume of 1.2 μl per 100 mm length); (CMA, Stockholm, Sweden) was used to connect the microinjection syringe pump to the inlet of the probe and the outlet of the probe to the sample loop were both accomplished with. The total dead volume of FEP tubing from probe to sample loop was approximately 6 μl. All connections of FEP tubing to the microinjection syringes, the probe and the other FEP tubing were accomplished by tubing adaptors (CMA, Stockholm, Sweden), which ensure tight, zero internal volume connections.

The on-line interface was performed with a six-port on-line injection valve (Omnifit Smart Actuator, Cambridge, UK). The on-line injection valve has an inert metal-free Teflon body designed for avoiding the contamination of magnesium. The connections and conduits were made of polytetrafluoroethylene (PTFE) connecting tubes (11 cm long × 1.0 mm i.d.) (Perkin-Elmer B019-1058). A 20 cm long delivery tube was used to connect the injection valve with a flame atomizer. A Perkin-Elmer Model 5100 PC atomic absorption spectrometer and a magnesium hollow cathode lamp (Perkin-Elmer) operating at 6 mA were used for the determination of magnesium. A personal computer was used to operate the AA WinLab software (Perkin-Elmer) for acquiring the magnesium absorption signal. To avoid the residual effect of the previous concentration, the sample loop was washed for 20 s by carrier solution for every change of sample loading.

3. Procedures

For characterization of the on-line microdialysis–FAAS system, we introduced the microdialysate sample directly into the injection valve in a continuous manner. Aqueous Mg standards were used to estimate the operational parameters (perfusion flow rate, sampling time, nebulizer uptake rate and detection time) instead of real samples from test animals.

The linearity of the calibration curve was evaluated from 0 to 320 mg l^{-1} by the on-line microdialysis–FAAS system. The detection limit (3σ) and quantitative limit (10σ) were carried out by seven-times determination of baseline noise. For long-term stability and precision testing, we inserted the microdialysis probe into the left-ear vein of rabbits, after which on-line sampling and detection was conducted every 1.5 min for 75 min (50 continuous measurements). The accuracy was checked by implanting the probe in the left-ear vein of three rabbits, the results of which were compared with the in vivo NNF method. In the in vivo NNF method, the microdialysis sampling was performed by perfusing the probe with different concentrations of Mg standard solution (4, 8, 16, 30, 40 and 50 mg l^{-1}) at a flow rate of 1.5 μl min^{-1}.

Adult male New Zealand White rabbits (2500–3000 g) were obtained from the Livestock Research Institute of the Republic of China (Tainan, Taiwan). The animal experiments were approved by an Animal Experiment Control Committee at Kaohsiung Medical University. These animals were specifically pathogen-free and were allowed to acclimate to their environmentally controlled quarters (25 °C and 12:12 h light–dark cycle) for at least 5 days and then fasted overnight prior to the day of experimentation. The rabbits were initially anesthetized with an anaesthetic solution (ketamine 45 mg kg^{-1}, xylazine 5 mg kg^{-1} and atropine 0.1 mg kg^{-1} body weight), and continued to be anesthetized with pentobarbital (10 mg kg^{-1} h^{-1}) throughout the experimental period. The microdialysis probe (CMA/20, 10 mm dialysis membrane) was implanted into the left-ear vein of each rabbit. The probes were perfused with a saline solution containing anticoagulant (20 IU ml^{-1} of heparin). The microdialysates collected over the first 2 h were discarded to prevent acute adverse effects on the animals from the surgical procedures. Each probe was connected to the on-line analytical system approximately 2 h after surgery. Mg was continuously monitored by the proposed on-line system every 1.5 min. Basal Mg levels were monitored for at least 9 min prior to Mg administration. When the sixth measurement was completed, $MgSO_4$ (40 mg kg^{-1} body weight) was injected into the right-ear vein of the rabbit. Mg was continuously monitored every 1.5 min for approximately 60 min.

4. Results and discussion

4.1. Optimization of the on-line microdialysis–FAAS system

The optimized operating conditions of this on-line microdialysis–FAAS system for the determination of diffusible Mg are summarized in Table 1. For characterizing the on-line microdialysis–FAAS system, the aqueous Mg standard solutions were directly sampled by a microdialysis probe and introduced into the flame atomizer in a continuous manner.

In this work a commercial metal-free probe (CMA/20) incorporating a 10 mm long microdialysis membrane was used to permit optimal sampling efficiency. In order to reduce the complexity of the matrix and to avoid Mg contamination, we perfused the implanted probe with an ultrapure saline solution (0.9% NaCl). This ultrapure saline solution is similar to blood in ionic strength and pH value. To obtain the optimal flow rate and sampling time, signals of aqueous 1 mg Mg l^{-1} standard solution were examined in terms of perfusion flow rate (10, 20, 30 and 40 μl min^{-1}) and sampling time (1, 2, 3 and 4 min) using the on-line microdialysis–FAAS system. As well known, in microdialysis the relative recovery will

Table 1
Optimized operating conditions of the on-line microdialysis–FAAS system for continuous monitoring of diffusible Mg

Microdialysis sampling	
Probe (CMA/20)	10 mm × 0.5 mm membrane, metal-free and cut-off at 20 kD
Perfusion solution	Saline (0.9% NaCl) solution (pH 7.2)
Perfusion flow rate	40 μl min^{-1}
Sampling time	1 min
On-line interface	
Nebulizer uptake rate	6 ml min^{-1}
Carrier solution	0.2% HNO_3
FAAS system	
Lamp type	Mg hollow cathode lamp
Wavelength	285.2 nm
Slit width	Low 0.7 nm
Lamp current	6 mA
Oxidant	Air (10 l min^{-1})
Fuel	Acetylene (2 l min^{-1})
Signal type	Peak area (integrated absorbance)
Read time	10 s
Washing time	20 s

decrease as the flow rate increases. Choosing lower flow rate would increase the relative recovery; however, the sampling time will be lengthened. Because the high free Mg extracellular concentration (about 0.7 mmol/l) [21], to obtain both sufficient microdialysate volume and rapid measurement, a 40 μl min^{-1} perfusion flow rate and 1 min of sampling time were therefore favorable for the determination of diffusible Mg using the on-line microdialysis–FAAS system.

Instead of off-line transporting the microdialysate to the FAAS, an injection valve was employed as an on-line interface. Coupling it to microdialysis for on-line monitoring should further demonstrate its versatility. The manifold for the on-line microdialysis coupled with FAAS is shown schematically in Fig. 1. In the system design, an on-line injection valve converts the continuous sampling stream of the microdialysis system into discrete samples. The microdialysate sample flows directly into the sample loop on the injection valve. In the loading step [Fig. 1(a)], the perfusion flow rate of 40 μl min^{-1} is converted into a 40 μl sample by the injection valve. After a 1 min loading period, the injection valve is switched to the injection position. The microdialysate sample trapped in the loop is injected into delivery tubing at timed intervals. In the injection step [Fig. 1(b)], the carrier solution is introduced into the sample loop to propel the microdialysate sample into the flame atomizer by a nebulizer uptake flow. Meanwhile, the spectrometer computer is actuated to read the atomic absorption signal for 10 s. In order to avoid the residual effect of the previous sample, the sample loop was washed by carrier solution for 20 s prior to the next loading step.

The effect of the nebulizer uptake rate on the absorbance of diffusible Mg was studied by adjusting the nebulizer regulator. Fig. 2 shows that the nebulizer uptake rate increases with a decrease of Mg absorbance. These results indicate that the lower the nebulizer uptake rate, the greater the absorbance rate. However, unstable signals were observed at a low nebulizer uptake rate. In this experiment, an optimal nebulizer uptake rate of 6 ml min^{-1} was selected according to the analytical stability and sensitivity. On the other hand, bubbles in the delivery tubing from the sample loop to the flame atomizer were observed using high-purity water as the carrier solution. The interference of bubbles could be eliminated using 0.2 % HNO_3 as the carrier solution.

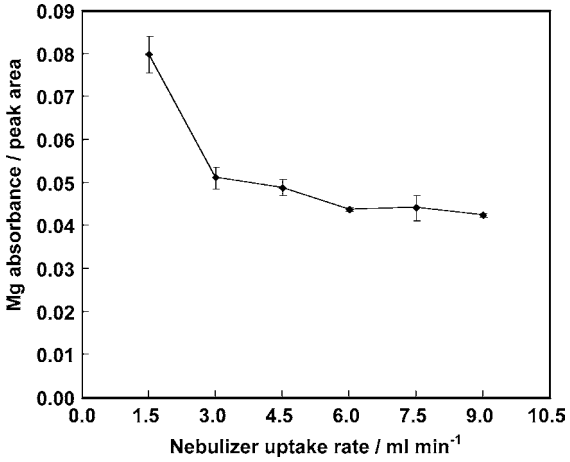

Fig. 2. Effect of the nebulizer uptake rate on the absorbance of 1 mg Mg l^{-1} standard solution using an on-line microdialysis–FAAS system. The nebulizer uptake rates of 1.5, 3.0, 4.5, 6.0, 7.5 and 9.0 ml min^{-1} were investigated. The error bars represent standard deviations ($n = 3$).

All parameters (Table 1) for the determination of Mg using FAAS were provided from the suggested values for the instruments except for signal type and read time. In this study, a small fixed volume of discrete microdialysate was introduced into the flame atomizer by the nebulizer uptake flow. A symmetrical or Gaussian-like signal type was formed during the process of Mg atomization. Therefore, the integrated peak area was evaluated as the absorbance of Mg. Furthermore, it is important to acquire the whole atomic absorption peak within a detectable time interval. The signal read time for an atomic absorption peak was 10 s.

4.2. Method validation of the on-line system

The linearity of the on-line microdialysis–FAAS system was evaluated from 0 to 320 mg l^{-1} Mg. The calibration graph was linear in the range of 0–300 mg l^{-1} Mg standard solutions using the on-line system were expressed by the regression equation (zero intercept): $A = 0.0068\,C$, $r > 0.9980$, where A is the absorbance, C the Mg concentration and r the correlation coefficient. Above 300 mg l^{-1}, the system showed a slight negative deviation from linearity. The detection limit, based on three times the standard deviation of the baseline noise ($n = 7$), was 0.53 mg l^{-1}. The quantitative limit based on 10 times the standard deviation of the baseline noise ($n = 7$) was 1.76 mg l^{-1}. The recovery tests for the on-line microdialysis–FAAS system were carried out at three different concentrations of samples spiked with Mg in rabbit blood samples.

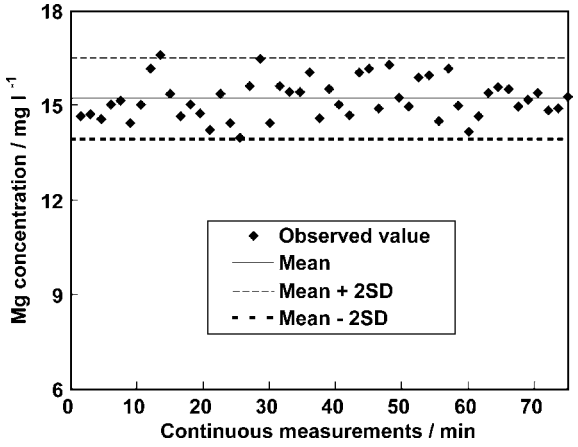

Fig. 3. Long-term stability of the proposed on-line microdialysis–FAAS system using continuous on-line sampling and detection every 1.5 min for 75 min (50 continuous measurements) in the blood of living rabbits.

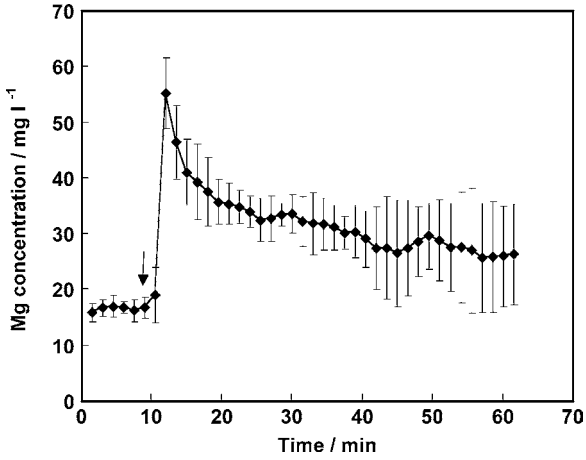

Fig. 4. The time course of Mg concentration in the blood of living rabbits following an experimental intravenous injection of Mg. (↓) Intravenous injection of 40 mg kg^{-1} body weight $MgSO_4$. Diffusible Mg in the blood of rabbits was measured using the on-line microdialysis–FAAS method. The error bars represent standard deviations ($n = 3$).

Mg levels in the blood of living rabbits were measured by the on-line microdialysis–FAAS system for long-term stability. The microdialysis probe was inserted into the living rabbits' left-ear vein, followed by on-line sampling and detection every 1.5 min for 75 min (50 continuous measurements). Fig. 3 shows that every measurement was in the range of ±2 standard deviation. The precision of the on-line microdialysis–FAAS system for 50 measurements was 4.1% R.S.D.

Because no certified values for the diffusible Mg content in rabbit blood were available, the accuracy of the proposed on-line method was examined by comparing Mg levels in the blood of three living rabbits with the results obtained from in vivo no net flux (NNF) method [23]. The in vivo NNF is the most commonly used quantitative microdialysis method, based on measuring the mass transport of the analyte across the dialysis membrane as a function of the perfusate concentration [23]. In this method, Mg is added to the perfusate at concentrations higher and lower than the expected true concentration. This generates a series of points that can be interpolated to determine the concentration of no net Mg flux, which represents the true concentration surrounding the probe. The comparisons between the on-line microdialysis–FAAS system and in vivo NNF method are shown in Table 2 and are in good agreement within experimental error.

Table 2
Diffusible Mg concentration (mg l^{-1}) in the blood of living rabbits as determined by the proposed on-line microdialysis method and in vivo no net flux method (Mean ± S.D., $n = 3$)

Rabbit	On-line microdialysis–FAAS system	In vivo no net flux method
1	17.8 ± 0.7	17.9 ± 0.5
2	16.1 ± 0.3	16.9 ± 0.3
3	16.9 ± 0.9	17.9 ± 0.3

Overall, the favorable analytical performance of the proposed method evaluated in terms of linearity, detection limit, spiked recovery, precision and accuracy indicates that an on-line microdialysis–FAAS system is appropriate for the continuous monitoring of diffusible Mg in the blood of living animals.

4.3. In vivo study

This on-line microdialysis–FAAS system was evaluated regarding the continuous monitoring of Mg in rabbit blood. In order to demonstrate the acute distribution of Mg in blood, an experiment involving the intravenous injection of Mg was performed. Mg was continuously measured in the blood of living rabbits using the on-line microdialysis–FAAS system after administrated with $MgSO_4$. Fig. 4 shows the concentration profile of Mg in the blood of living rabbits. Basal microdialysate levels of Mg (16.6 ± 0.4 mg l^{-1}, $n = 6$) were determined at 1.5 min intervals for 9 min. Forty milligram per kilogram body weight of $MgSO_4$ was then intravenously injected into the right-ear vein of each rabbit. Following the administering of Mg, the average time for the initial rise was 2.0 ± 0.9 min ($n = 3$). The average concentration of maximum Mg during stimulation was 55.3 ± 6.4 mg l^{-1} ($n = 3$). The average blood Mg concentration reached a maximum value at 3 min post-injection, approximately 3.3-fold higher than the basal level and the value was still 1.6-fold higher than the base line at 60 min after $MgSO_4$ administration.

5. Conclusions

In this work, a novel method involving on-line microdialysis sampling and FAAS analysis for the in vivo monitoring of Mg concentrations in the blood of living rabbits was

developed. On-line microdialysis that provides direct, in situ, dynamic and continuous sampling simplifies the pretreatment of biological samples. On-line microdialysis sampling coupled with FAAS makes it simple and fast for continuous trace metals monitoring in living organisms. This on-line analytical technique developed may also be employed to investigate the distribution of trace metals in tissues, organs or biological fluids of living organisms.

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