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Original Article

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Membrane-Introduction Mass Spectrometry Analysis of Desflurane, Propofol and Fentanyl in Plasma and Cerebrospinal Fluid for Estimation BBB Properties

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A possibility to use the Membrane-Introduction Mass Spectrometry (MIMS) with membrane separator interface has evolved into a powerful method for measurement of anaesthetic agents absolute concentration in blood plasma and cerebrospinal fluid for the study of blood-brain barrier (BBB) properties. Recent advanced a new membrane material was used for drug concentration measurement in biologic fluids. A hydrophobic membrane was used in the interface to separate anaesthetic agents from biological fluids: inhalational anaesthetic desflurane,hypnotic propofol, analgesic fentanyl. The selective detection of volatile anesthetic agents in blood does not require long-term sample processing before injecting the sample into mass-spectrometer interface, in contrast to chromatographic methods. Mass-spectrometric interface for the measurement of anaesthetic agent concentration in biological fluids (blood plasma and cerebrospinal fluid) is described. Sampling of biological fluids was performed during balanced inhalational (desflurane, fentanyl) anaesthesia and total intravenous (propofol, fentanyl) anaesthesia.

Key words: membrane, mass-spectrometer, anesthesia, desflurane, fentanyl

INTRODUCTION

Chromatographic-mass-spectrometric methods for the analysis of biological fluids require long-term sample processing which is associated with single extraction procedure or head space-gas chromatography with solid phase microextraction last time. To address practical problems of anaesthesiology (including monitoring of anaesthesia depth and assessment of blood-brain barrier permeability), development of an express method is relevant to allow measuring concentration of anaesthetics in biological fluids in clinical practice, i.e. without long-term multi-stage sample processing.

Currently, methods exist for absolute concentration measurement of wide spectrum of organic compounds dissolved in water. Membrane-Introduction Mass Spectrometry (MIMS) method demonstrate high analytical potential in chemical analysis designed for selectivity and sensitivity [1]. The MIMS sample introduction interface is coupled to a quadrupole mass-spectrometer for analyte detection and determination [2]. A limit of detection for drug concentration as good as 10^{-8}%~10^{-7}% (10^{-5} μg/l) was achieved [3]. Disadvantages of MIMS include longer response time compared to capillary injection directly into the ion source of mass-

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spectrometer, dependence of membrane characteristics on the temperature, and selectivity (transmission coefficient) for various compounds [4, 5].

MATERIAL AND METHODS

All clinical studies were approved by the administration of the Kirov Medical Academy (MMedA).

All patients gave written consent to participate in the investigation.

The Mass Spectrometry System

In the present work, a mass-spectrometric membrane separator interface with a 150 μm polydimethylsiloxane membrane (SSP-M100, Specialty Silicone Products Inc., Ballston Spa, NY, USA) was used for the first time to investigate concentration of anaesthetic agents in biological fluids. Anaesthetic agents crosses the membrane by a three-step process (1. Absorbs into the membrane, 2. Diffuses, 3. Evaporates into ion source of the mass spectrometer) named pervaporation. Using mechanism pervaporation, the membrane blocs plasma from passing through membrane and allows anaesthetic agents to pass through the membrane. A quadrupole electron-impact mass-spectrometer (QEIMS) was used in the study. The QEIMS (Prisma Plus, Pfeiffer Vacuum, Germany) consists of an electron impact ion source, a quadrupole mass analyzer and a dual ion current detector (A Feraday cup for direct measurement of the ion current and a secondary electron multiplier (SEM)). In this work we have used a selected ion monitoring (SIM) mode and SEM Bargraph Cycles mode. A titanium membrane was used to fix the membrane on the aperture of the interface flange 10.0 mm in diameter. A system of differential pumping provided a pressure drop of 1000 mbar — $3.0 \cdot 10^{-2}$ mbar — $4.0 \cdot 10^{-6}$ mbar, where $3.0 \cdot 10^{-2}$ mbar presented pressure in the differential chamber- $4.0 \cdot 10^{-2}$ mbar and 10^{-6} mbar – pressure in the mass-spectrometer chamber, respectively. Interface design allowed heating it to 45°C. The mass-spectrometer chamber and the differential pumping chamber were vacuumed using a turbomolecular pump and the first (molecular) pumping stage of the same pump. Pumping rate for the mass-spectrometer chamber and the differential chamber was 60 l/s and 20 l/s, respectively. These chambers are separated by a diaphragm 100 μm in diameter (Pfeiffer Vacuum, Germany).

Anesthesia

Before induction of anaesthesia, all patients were pre-oxygenated for 1.0 min. In the target-controlled infusion (TCI) group anaesthesia was induced with propofol (Fresenius) 1.0 mg kg^{-1} and fentanyl 5.0 μg kg^{-1} and patients received propofol 2.0 mg kg^{-1} and a fentanyl 5.0 μg kg^{-1} for iduction of anaesthesia. One min after starting anesthesia, rocuronium bromide 0.6 mg kg^{-1} was administered in both groups to achieve muscle relaxation. After tracheal intubation, all patients were ventilated mechanically by using a constant fresh gas 7.0 l min^{-1}. During total intravenous anaesthesia, infusion regimen was used controlled by the target propofol ($C_{12}H_{18}O$) concentration in blood. A concentration of 2 μg/ml was selected and maintained by a syringe pump (B|Braun) under TCI regimen controlled by TCI program - "Stanpump" [6]. Anesthesia was maintained by TCI of propofol 3.0 mg kg^{-1} and fentanyl 0.1 mg over a 20 min period in the TCI group and with desflurane 6.0±0.5 vol% minimum alveolar concentration (MAC). The balanced inhalational anesthesia was performed with the inhalational anesthetic agent desflurane (Baxter Healthcare) in a dosage of MAC. Sampling was performed *in vivo* during balanced inhalational anaesthesia with desflurane at a concentration of 6.0% vol in the breathing circuit (BC) of the inhalational anaesthesia machine (IAM). At the end of surgery, anaesthesia was stopped and the patients' lungs were ventilated with fresh gas 7.0 l min^{-1}.

A 10.0 μl sample was injected directly onto the interface membrane into a closed chamber located immediately above the membrane. Blood and cerebrospinal fluid samples were pre-centrifuged for 10 min at a 5000 rpm rate.

RESULTS AND DISCUSSION

Fig. 1 shows a section of the MIMS mass-spectrum for gas mixture dissolved in blood plasma, obtained using the membrane interface. Mass peaks of 51 m/z, 101 m/z, 149 m/z correspond to desflurane ($C_3H_2F_6O$) [7]. These peaks were absent in the plasma samples of healthy volunteers. Blood samples were drawn from peripheral vein (PV) and from surgical wound in the chiasmo-sellar area of the brain (CSAB) during adenomectomy of pituitary tumor. The ratio of desflurane concentration in PV (Plasma (PV)) and in CSAB (Plasma (PV)/Plasma (CSAB)) was 5.4±1.4. The indicated difference in concentration is due to the properties of the BBB [8]. BBB lies between endothelial cells lining blood capillaries in the brain; therefore, the anaesthetic agent concentration in the blood from surgical wound in CSAB represents its concentration after passing through BBB. The ratio of desflurane concentrations in PV and in cerebrospinal fluid (CSF) - Plasma (PV)/CSF=1.25±0.2. Sampling of cerebrospinal fluid was performed during ventriculoperitoneal shunting (VPS) associated with the necessity to compensate increased intracranial pressure. Because of increased intracranial pressure, CSF is accumulated in intercellular space, which leads to vasogenic brain

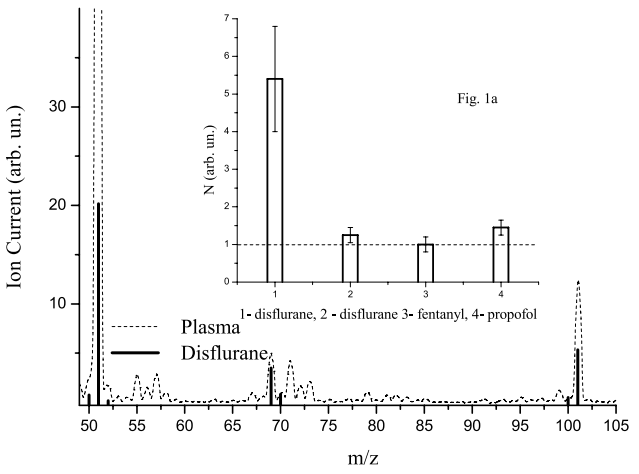

Fig. 1. Mass-spectrum of desflurane and desflurane in the plasma that was taken during balanced inhalational anaesthesia, obtained using membrane separator interface. Fig. 1a Ratio of drug concentrations in a peripheral vein and in the brain where N (arb. un.) is the ratio concentrations of the anaesthetic agents in PV and in chiasmo-sellar area (cerebrospinal fluid), respectively (see details in the text).

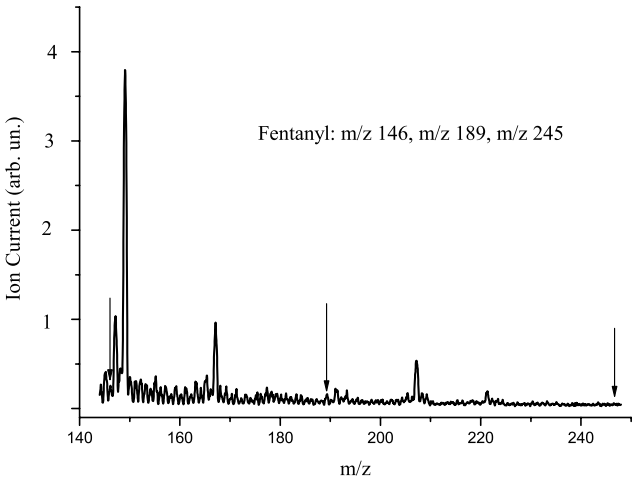

Fig. 2. Mass-spectrum of fentanyl in the plasma that was taken during balanced inhalational anaesthesia, obtained using MIMS.

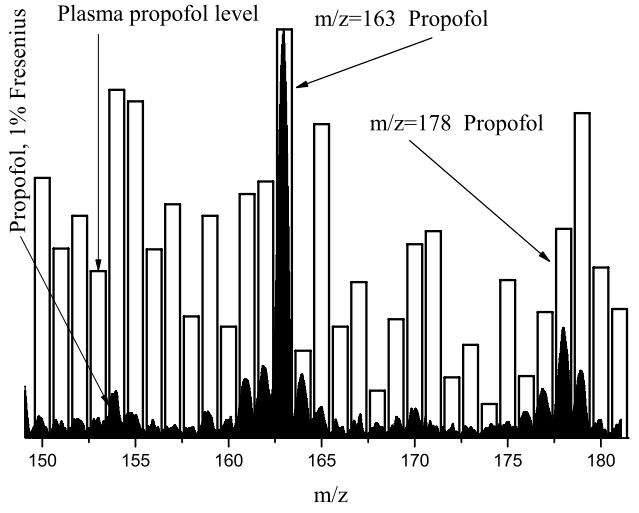

Fig. 3. Mass spectra of propofol (Fresenius Kabi) and blood plasma sample taken during total intravenous propofol-fentanyl anesthesia, measured using MIMS.

edema causing an increase in BBB permeability. The difference between Plasma (PV)/Plasma (CSAB) and Plasma (PV)/CSF is mainly due to the fact that pituitary adenoma (when Plasma (PV) and Plasma (CSAB) samples were taken) does not lead to increase in BBB permeability, in contrast to CSF sampling during VPS for intracranial pressure compensation. The results were obtained during 12 anaesthesia procedures. It should be noted that a BBB properties study, i.e. assessment of drug ratio in brain tissue and in blood - logBB=Brain/Blood, where Brain is drug concentration in brain tissue and Blood is blood concentration, respectively, requires expensive and long-term studies since brain tissue samples are taken from the patients who died during the surgery [9, 10]. These results are used in pharmacology and for developing a mathematical model for analytical calculations of logBB [11]. To find the proportionality factor for logBB and Plasma (PV)/Plasma (CSAB), additional studies are required to increase statistical significance of the obtained results.

Throughout the anaesthesia opioid analgesic fentanyl (State Plant for Drugs Manufacturing) ($C_{22}H_{28}N_2O$) 0.1 μg/ml was administered every 20 minutes. The fentanyl mass-spectrum in the plasma is shown in the Fig. 2. Estimation of the ratio of fentanyl concentration in PV and CSAB was performed in the present study. Plasma (PV)/Plasma (CSAB)=1.0±0.2 (see Fig. 1a) and, therefore fentanyl enters the central nervous system (CNS) without obstruction, which is consistent with the result obtained in rats [12].

Fig. 3 presents the MIMS mass spectrum of propofol (Fresenius Kabi). The propofol emulsion was applied directly onto the interface membrane of the mass spectrometer. Fig. 3 also shows the mass spectrum of a blood plasma sample taken during total intravenous propofol fentanyl anesthesia.

The mass-spectrum of blood samples taken during the total intravenous anaesthesia with propofol and fentanyl is shown in the Fig. 4. Intravenous hypnotic propofol is practically insoluble in water, but is highly soluble in lipids, therefore this drug is administered intravenously as an emulsion that includes: 10% soya bean oil, 1.2% purified egg phospholipids (emulsifier), 2.25% glycerol, water, and sodium hydroxide. Emulsified propofol formulation was marketed in 1986 and currently is a

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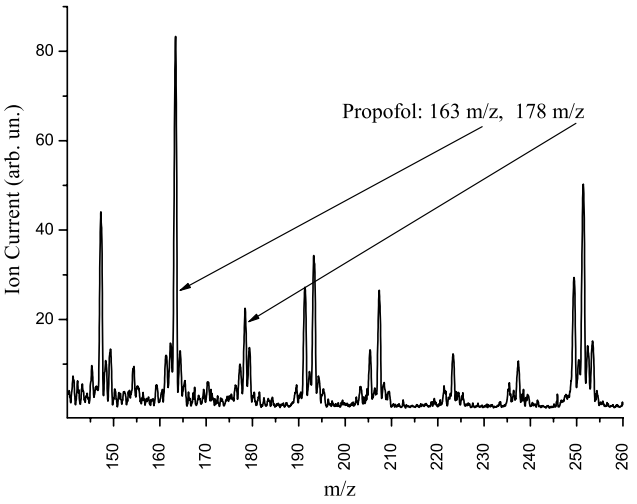

Fig. 4. Mass-spectra of propofol in the plasma that was taken during total intravenous propofol-fentanyl anaesthesia, obtained using MIMS.

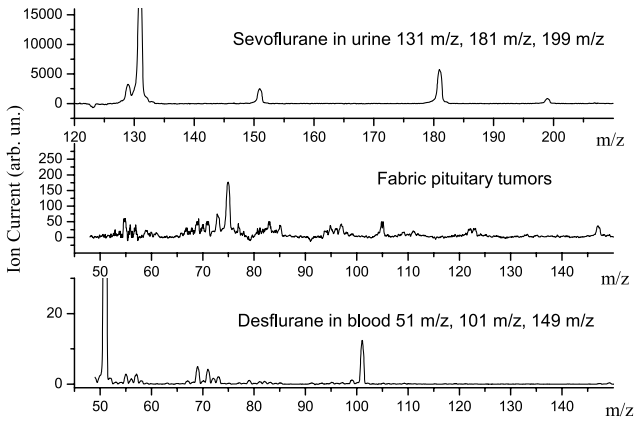

Fig. 5. "UP" -The MIMS Mass spectrum of sevoflurane -fentanyl in the urine that was taken during sevoflurane-fentanyl anaesthesia. "Middle"- the MIMS mass spectrum of evaporation through a membrane fabric (tissue) pituitary adenoma. "Bottom" -the MIMS mass spectrum of desflurane in the blood that was taken during desflurane-fentanyl anaesthesia.

hypnotic of choice for inhalational or intravenous anaesthesia. The propofol solution in the Intralipid drug for parenteral (intravenous) nutrition (Fresenius-Kabi) was prepared to serve as an internal standard; its composition is consistent with that of the propofol dissolution medium (Fresenius-Kabi). Based on four measurements of propofol concentration in plasma, it was equal to the specified target concentration of propofol in the program "Stanpump" with accuracy of at least 5.0%. High solubility of propofol in the lipids allows its easy entrance to the CNS, which explains its rapid action.

In the present study, blood samples were taken from PV and CSAB. Propofol concentration measurements in plasma for these two samples were performed using the membrane separator interface. The propofol concentration in the chiasmo-sellar area was Plasma (PV)/Plasma (CSAB)=1.45±0.2 less than in peripheral vein (see Fig. 1a. Estimation of the ratios for propofol concentrations was performed using fragmentation mass peaks of propofol 163 m/z (see Fig. 4). On conclusion, is presented 3 the mass spectrum obtained by using MIMS method sequentially on one membrane. Fig. 5 "up" shows the MIMS mass spectrum suvoflurana in urine. Urine was carried out during sevofolurane-fentanyl anesthesia. Fig. 5 "middle" shows the MIMS mass spectrum of evaporation through a membrane fabric (tissue) pituitary adenoma. Fence tissue occurs during removal of pituitary adenoma (anaesthesia — desflurane-fentanyl). Fabric adenoma was maintained in air for 15 min and then it was fixed on the membrane surface of the interface. During this time desflurane evaporates from adenoma fabric and desflurane mass-peaks was absent. Fig. 5 "bottom" shows the MIMS mass spectrum of desflurane in blood. Blood sampling was carried out during a desflurane-fentanyl anesthesia. These results demonstrate the absence of "memory" effect at the MIMS technique.

CONCLUSION

As a result of the performed measurements (up to 30 cycles) no degradation of membrane properties was found. Duration of one measurement is 1 min. Membrane interface is easy to use and maintain and has a potential for use in practical anaesthesiology for express analysis of anaesthetic drug concentration in plasma and CSF.

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