Electrochimica Acta 138 (2014) 360–366

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Electrochemical impedance studies on the interaction of midazolam with planar lipid bilayer

Kumaravel Mallaiya[a,*], S. Rameshkumar[a], S.S. Subramanian[a], S. Ramalingam[b], T. Ramachandran[c]

[a] Department of Chemistry, PSG College of Technology, Peelamedu, Coimbatore 641 004, India
[b] PSG Institute of Medical Science and Research, Coimbatore 641 004, India
[c] Department of Chemistry, Amrita Vishwa Vidyapeetham University, Coimbatore 641 112, India

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 7 June 2014
Accepted 7 June 2014
Available online 30 June 2014

Keywords:
Midazolam
BLM
Nonspecific action
Impedance spectroscopy
Capacitance

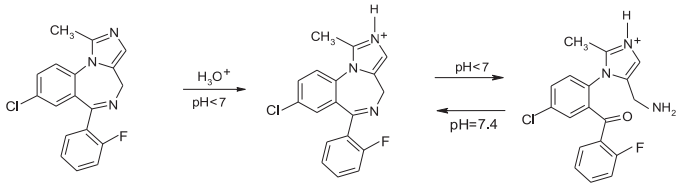
ABSTRACT

This work presents the non-specific action of midazolam (MDZ) on planar bilayer lipid membranes (BLMs), studied by following the dose-dependent changes in the electrical characteristics of BLMs. MDZ is found to interact with the polar regions or the hydrocarbon core of the membrane depending on whether it exists in charged or neutral form. The extent of these interactions depends on the ionic strength of the medium supporting the membrane, which influences the proportions of the charged and neutral forms of MDZ present in the medium. The observed electrical characteristics of the membranes are a consequence of competitive effects brought about by these drug species on both regions of the membrane. The drug is observed to impart a stabilizing effect at low doses and a destabilization above a saturation level at the polar regions of the lipids. These results demonstrate that the level of MDZ necessary for the destabilization and fluidization effect on biomembranes is close to that obtained after a single dose, and that its continuous administration in the spinal cord is neurotoxic.

1. Introduction

Midazolam (MDZ) is an ultra short-acting tricyclic benzodiazepine used in anesthetic practice. When compared to the traditional bicyclic benzodiazepines, it exhibits better local tolerance, faster onset of action, faster plasma clearance, and a shorter half-life elimination (1.7-2.4 hours) with no active metabolites. Presently, MDZ in the form of its hydrochloride is the most widely used intravenous benzodiazepine [1–5].

Chemically, MDZ is 8-Chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo-[1,5-a] [1,4]-benzodiazepine ($C_{18}H_{13}ClFN_3$), and it differs from most of the "traditional bicyclic benzodiazepines" in having a nitrogen atom in its additional ring structure. This nitrogen atom is not sufficiently basic to be protonated at physiological pH, but it is basic enough to yield water-soluble salts when treated with strong acids. Midazolam hydrochloride ($MDZH^+Cl^-$) displays a pH dependent opening of the benzodiazepine ring below pH ≈ 4.0, but following injection, at physiological pH, the imidazole ring closes, resulting in a highly lipid-soluble drug [3–6].



The exact mechanism and sites of action of benzodiazepines responsible for their pharmacological effects are still under study. Benzodiazepines are proposed to act on the subunits of $GABA_A$ receptors at the postsynaptic neurons and to augment the activity of the inhibitory neurotransmitter γ-amino butyric acid [7–12].

The clinical use of these drugs involves a compromise between their benefits and toxicities. Many studies using animal models showed that spinally administered MDZ induces severe neurotoxicities [13–17], but many reports contradict these results [18–24], which leads to the present work.

Severe neurotoxicity has been reported with a single dose of intrathecal MDZ administration in rabbits [13,14]. Chronic subarachnoid administration of both preservative-free and commercial preparations of MDZ in rats have been shown to be

Abbreviations: BLM, Bilayer Lipid Membrane; MDZ, Midazolam; $MDZH^+Cl^-$, Midazolam Hydrochloride; FDD, Frequency Dependent Dispersion.

* Corresponding author.
E-mail addresses: mkvteam.research@gmail.com, mkv@che.psgtech.ac.in (K. Mallaiya).

http://dx.doi.org/10.1016/j.electacta.2014.06.038
0013-4686/

neurotoxic with significant decreases in cell number and a tendency towards a higher mean cell volume, with a loss of small neurons confirming neuronal death [15].

On the other hand, long-term intrathecal infusions of midazolam in humans have been shown to cause no neurotoxicity [18]. After MDZ administration, sections of rat spinal cord did not show any morphological changes suggestive of MDZ-induced neurotoxicity [19]. MDZ had minimal neurotoxicity in rat spinal cords, only at a higher dose of 3 mg/kg [20]. Reports continued to show no significant differences in the histological changes in neural tissues, despite repeated applications of MDZ in the subarachnoid space [21].

MDZ, which induces anesthesia in humans at intravenous doses of 0.3 mg/kg, did not anesthetize cats at doses of 20 mg/kg [22]. Spinally administered MDZ, even in large doses, did not cause acute neurotoxicity or inflammation of the spinal cord of cats [23]. Addition of MDZ to human cerebrospinal fluid in saline neither decreased the pH below 7.0 nor reduced transparency [24]. Hence, the results of this in vitro study also suggested that clinically useful doses of intrathecal or epidural MDZ are not neurotoxic.

Another interesting result was the finding that MDZ provides protection against opioid (fentanyl) neurotoxicity [25]. Narcotics in large doses cause brain damage in rats, which is attenuated by naloxone, MDZ, and phenytoin, which are respectively a narcotic antagonist, a sedative, and an antiepileptic drug [25].

Though receptor-specific interactions are present, the possibility of site-nonspecific interactions on the lipid core cannot be excluded [26,27]. Since the bilayer construct is a common feature for all types of cell membranes, nonspecific disturbances to the membrane could occur to all cells, including target cells. Such site-nonspecific interactions (drug-lipid interactions) may be the cause of undesirable side effects such as neurotoxicities [26,27]. The diversity of the structures of molecules that can act as CNS depressants led to the argument that anesthetic-receptor interactions could also be nonspecific [27]. There has been a long-standing controversy over whether membrane lipids or proteins are the targets for general anesthetics. Lipid/protein interface is shown to be the anesthetic target, rather than protein or lipid alone [28].

The interaction of drugs with cell membranes can be studied using model membrane systems that mimic the lipid bilayer architecture of the cell membranes such as lipid vesicles, bilayer lipid membranes, supported bilayer lipid membranes etc., [29–33]. Of these, the bilayer lipid membrane system is extensively used as an experimental model for biomembranes [34,35]. Human myelin consists of about 80% lipids and only 20% proteins [36], and hence artificial lipid bilayers serve as the best models of nerve cell membranes.

Only a few studies have been conducted on the nonspecific interaction of benzodiazepines with model membranes [37–39], and little work has been done on MDZ-BLM interaction. Benzodiazepines bind to specific receptors, but also interact nonspecifically with the lipid part of the membrane. Flunitrazepam was found to localize at the polar region of the bilayer [37]. The extent of interaction and localization of benzodiazepines at the lipid-water interface depends on the nature of the medium, the membrane composition and the chemical structure of the drug [38]. On the other hand, benzodiazepines were shown to localize at the hydrocarbon core of the membrane [39]. The above two results give two different views on the interaction of benzodiazepines with model membranes, and the implication of these results will be discussed.

Studies on benzodiazepine partitioning between octanol and a buffer at physiological pH showed that lipid solubility partly determines the extent of benzodiazepine distribution in vivo, which in turn is a major determinant of the duration of clinical action after a single dose [40]. Nonspecific interactions have important consequences for a wide variety of biological functions. This type of interaction may mediate several effects of benzodiazepines observed at global concentrations above their affinity for their specific receptors [40]. It is well known that the black lipid membranes used as a model membrane in our studies can be very well characterized using Electrochemical impedance spectroscopy (EIS) [41–48]. Moreover, the impedance technique has provided a non-invasive means of characterizing the electrical properties of many systems [41,48]. This paper describes the real time characterization of MDZ - bilayer membrane interactions using electrochemical impedance spectroscopy.

2. Materials and methods

Midazolam in its hydrochloride form, obtained from Neon Laboratories Ltd, India, was used for the studies. Egg lecithin containing greater than or equal to 99% L-α-Phosphatidylcholine (Sigma Aldrich) was dissolved in chloroform (5 mg/mL) and used as the BLM forming stock solution. The BLM-forming dispersion was prepared by evaporating 100 μL of the stock solution in a 2 mL screw-cap tube under nitrogen atmosphere and dissolving the resulting lipid film in 200 μL of n-decane (Merck, Germany).

The BLMs were formed using a chamber, constructed indigenously by fixing a 1mm-thick PMMA septum containing a 1 mm diameter aperture between the 5 mL cavities drilled into two halves of the PMMA block cut from an 85 × 60 × 22 mm block. Stirring device, faraday cage and Ag/AgCl electrodes were fabricated following standard procedures [30–32,49–52,34]. A vibration isolated platform (MINUSK USA) was used to arrest the floor vibrations.

The BLMs were formed by employing the standard procedure proposed by H.T. Tien [30]. The required bathing solution (1 M or 0.1 M NaCl solution at pH = 7) was added to the BLM chamber after preconditioning the aperture with about 2 μL of the dispersion, and approximately 5 μL of the BLM forming solution was delivered directly over the aperture. In a stabilization time of 30 minutes, at an applied DC potential of 40 mV, the phospholipid molecules formed a lipid bilayer membrane spontaneously through self assembly. In the stabilization time, the capacitance of membrane increases continuously due to the self assembly of phospholipid molecules into bilayer phase by excluding solvent molecules and reaches a steady value which is in the same order reported in the literature [30,33,37,48,49].

The electrochemical impedance spectra of the BLMs were recorded using a Potentiostat (PARSTAT 2273, Princeton Applied Research–USA). After the stabilization of the membrane, the impedance measurements were carried out in the frequency range 1 MHz to 10mHz at the open circuit potential by superimposing a sinusoidal AC signal of small amplitude 25 mV. Data acquisition was performed utilizing PowerSuite software and analyzed using ZSimpWin 3.21 software.

After every drug dose, a stabilization period of 30 minutes was allowed for the drug to equilibrate between the lipid bilayer and aqueous phases. In accordance with earlier reports, each experiment was repeated to a minimum of 5 to 7 times to confirm the trend and to avoid errors.

3. Results and discussion

The dose-dependent changes induced by MDZ in the electrical characteristics of the bare and drug-doped membranes were studied and compared.

3.1. Electrical characterization of interaction of MDZ with BLM

The Nyquist plots obtained for bare and drug-doped BLMs in 1 M and 0.1 M NaCl bath solutions are shown in Fig. 1a and 1b