

Stability of diluted neuromuscular blocking agents utilized in perioperative hypersensitivity evaluation

To the Editor,

Hypersensitivity reactions may occur during surgery, with varying incidence from 1:4000 to 1:20 000.¹ One of the most common identifiable causes of hypersensitivity reactions during anesthesia is neuromuscular blocking agents (NMBAs).² Patients who have experienced a hypersensitivity reaction during anesthesia should undergo an allergy evaluation including skin testing to agents

exposed during surgery including NMBAs.² Skin testing to NMBAs has not been standardized, and variable concentrations are utilized through countries and institutions.²⁻⁶ We sought to determine the refrigerated stability of various diluted NMBA concentrations commonly used for skin testing.

Neuromuscular blocking agents used in the study included rocuronium bromide, cisatracurium, vecuronium bromide,

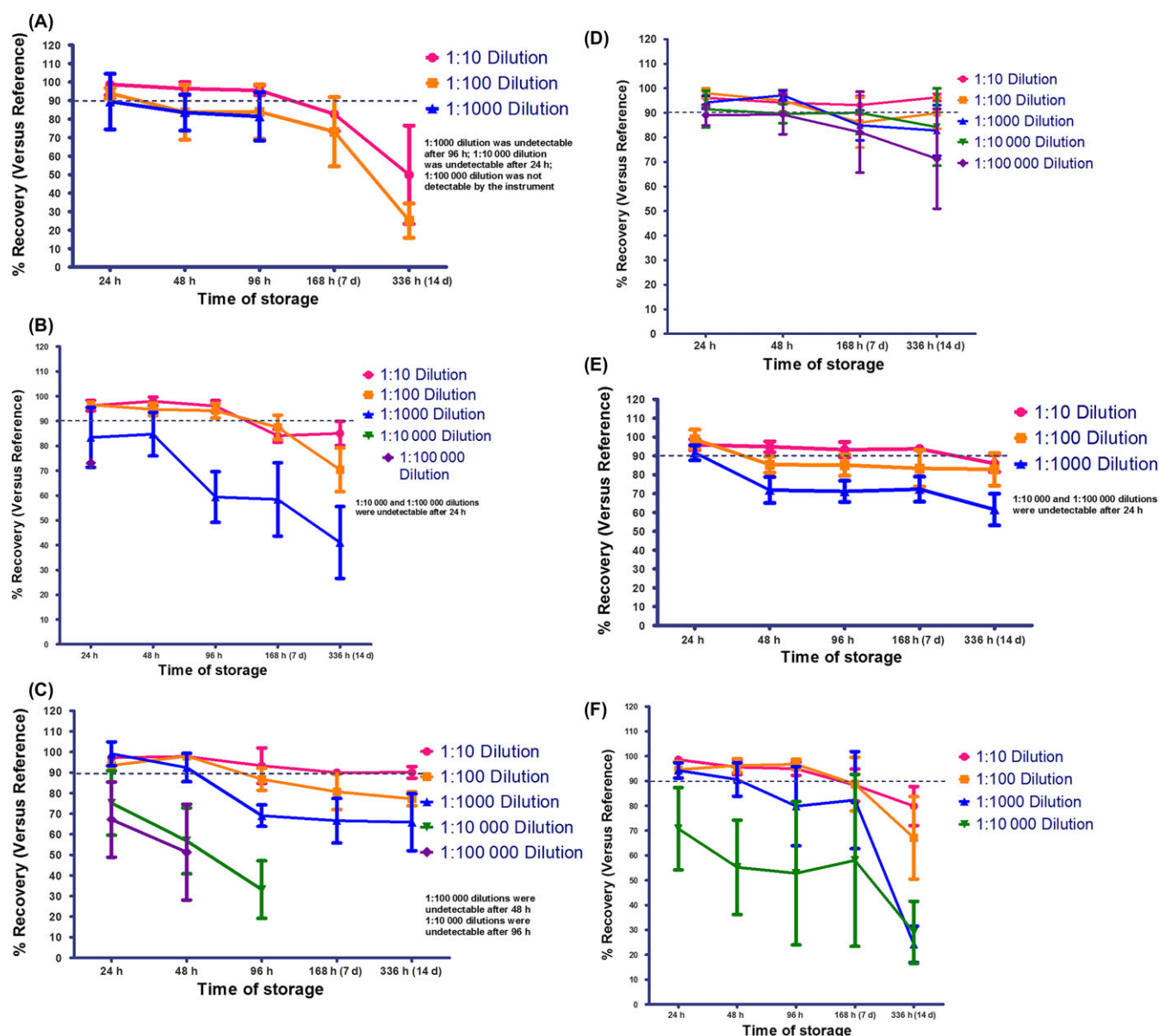


FIGURE 1 Percent recovery of dilutions of (A) atracurium, (B) cisatracurium, (C) pancuronium, (D) rocuronium, (E) succinylcholine, and (F) vecuronium stored for 14 d under refrigerated temperatures [Colour figure can be viewed at wileyonlinelibrary.com]

pancuronium, succinylcholine, and atracurium. Dilutions were prepared in NaCl for injection, United States Pharmacopeia (USP) (0.9%), and reconstitutions (vecuronium bromide in sterile water for injection). All experiments were completed prior to the expiration dates of the drug products. Calculations and graphical presentations were made using GraphPad Prism software, v 5.03, La Jolla, CA, USA.

Concentrations of NMBA were monitored by liquid chromatography-mass spectrometry (LC-MS/MS) for a period of 14 days. For each drug, a set of serial dilutions ($n = 5$ for each dilution) were prepared from the commercial product corresponding to 1:10, 1:100, 1:1000, 1:10 000, and 1:100 000. Dilutions were stored in a laboratory refrigerator (2–8°C) as per the United States Pharmacopeia (USP) guidelines.⁷ On days that samples were processed, a fresh cohort of solutions was prepared for the drug being analyzed. These served as the reference standards (% relative standard deviation 0.039–4.31) for the LC-MS analysis from that sampling day. Samples were collected after 1, 2, 4, 7, and 14 days of storage. Percent recovery for each stored sample was calculated against the freshly diluted reference dilutions of equal magnitude.

The results of the recovery calculations are shown in Figure 1. Each data point is shown as an average of five samples, and error bars represent standard deviation. The beyond-use date (BUD) point is defined as a loss of >10% potency, as compared to the reference solution.⁸ Not all drugs were detected at all dilution levels prepared, or no drug was detectable after 24 hours of storage; thus, these dilutions are recommended for immediate use. In some cases, the drugs were not detectable at all in the highly diluted concentrations; thus, their stability cannot be determined at these levels.

Variable dilutions of NMBA are commonly utilized for skin testing.^{2–6} We performed a stability study using an LC-MS/MS on all available published NMBA concentrations^{2–5} diluted with normal saline utilized for skin testing over a 14-day period. A previous manuscript has recommended storing diluted NMBA up to 3 months at 4°C.⁹ Based on our findings, we recommend storing dilutions of 1:10 for up to 48 hours and not storing dilutions of 1:1000 beyond 24 hours due to the risk of compound degradation. Higher dilutions should be utilized immediately.

All the drugs included in this study can be diluted, with confidence in stability at a 1:10 dilution and stored under refrigeration for 48 hours. Some can be stored for up to 96 hours at 1:10 dilution, namely atracurium, cisatracurium, and vecuronium. For the 1:100 dilution, all drugs in this study can be stored refrigerated for 24 hours, and most can last 2–4 days. When the dilution rises to 1:1000, some drugs cannot be stored at all (atracurium and cisatracurium), and must be used immediately. Beyond the 1:1000 dilution, these compounds have stability that limits them to immediate use, as their potency had fallen below 90% by the time of the 24-hour sampling. The more dilute preparations have a higher ratio of water: drug, thus expediting the ester hydrolysis that is likely responsible for the majority of these compounds' degradation. Furthermore, some high dilutions could not be detected at all at the 1:100 000 level, despite the use of the high-resolution LC-MS system.

We acknowledge some limitations to our study, as we did not include dilutions with injectable water and other variables that may have affected molecular adsorption such as types of syringe and storage vials/flasks; however, we utilized commonly used materials for dilutions used in clinical practice. Mivacurium was not included in the study as this medication was not available at our pharmacy and other stock concentrations not used by our institution were not included in our study (Atracurium 1 mg/mL, Succinylcholine 10 mg/mL, Vecuronium 4 mg/mL).

All NMBA were stable for at least 48 hours in the 1:10 dilution and for 24 hours in the 1:100 dilution. When the dilution magnitude is increased, the stability of the drugs at these concentrations generally decreases. The more dilute preparations have a higher ratio of water: drug, thus expediting the ester hydrolysis that is likely responsible for the majority of these compounds' degradation.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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[Correction added on 05 November 2018 after first online publication: One of the author names was previously incorrect and has been corrected in this version.]

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microRNA-146a is linked to the production of IgE in mice but not in atopic dermatitis patients

To the Editor,

Atopic dermatitis (AD) is a chronic and complex inflammatory skin disease characterized by pruritus and eczema. Its incidence has increased rapidly during the last 50 years and nowadays it affects up to 20% of children and 5% of adults in developed countries.^{1,2} According to genetic and molecular studies, AD manifestation is associated with skin barrier weakness and susceptibility to sensitization of the immune system to environmental and/or intrinsic factors acting in the skin. Although AD is usually treated as a singular condition, it is

mechanistically a very complex disease and at least two AD subtypes can be distinguished: allergic type (also named as extrinsic), affecting approximately 75% of patients, is characterized by a strong type-2-cell-mediated immune response and remarkably high levels of serum immunoglobulin E (IgE), whereas nonallergic type (also named as intrinsic) is characterized by normal IgE levels.^{1,3} All processes contributing to the development of AD, including innate and adaptive immune responses, can also be influenced by microRNAs.⁴ Among other immune-regulatory microRNAs (miRNAs), miR-146a has been shown to

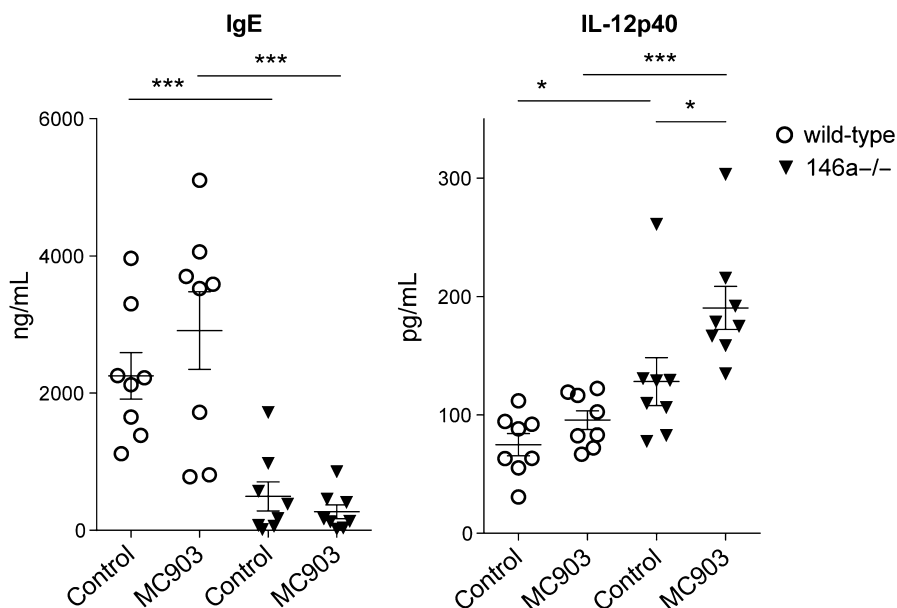


FIGURE 1 Decreased serum IgE levels and increased serum IL-12p40 levels in miR146a^{-/-} mice. Serum IgE and IL-12p40 levels in MC903 and control treated wild-type and miR146a^{-/-} mice. Results from one of two independent experiments are shown. Data are presented as mean with SEM, unpaired student *t* test, **P* < 0.05, ****P* < 0.001