Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

The effects of some organic solvents on the modified Ellman procedure for the assay of cholinesterases

Tugba Ercetin**, Acelya Mavideniz**, Karar Tawfeeq Shukur, Ali Khosravi, Bahamehr Riazi, Fatemeh Salamati, Houra Shahinfar, Mohammad Najjaran, Muzahem Imad Ahmed, Nasim Maher, Negar Khajeh, Nezhla Kazemi Davdar, Salva Shahinfar, Hayrettin Ozan Gulcan*

Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

Abstract

Ellman's method is the most acknowledged procedure in order to determine the cholinesterase inhibitory potential of substances. It is a quick and high-throughput screening method. Therefore, it is widely used to measure the activities of original molecules and mixtures in aqueous solvents through a UV method. Regarding that many original or known substances as well as mixtures require some organic solvent to guarantee dissolution within this methodology, it becomes critical to be aware of the inhibitory potential of organic solvents. From this perspective, within the present study, it was aimed to screen the inhibitory potential of some organic solvents on human acetylcholinesterase and human butyrylcholinesterase enzymes. The results displayed the potent inhibitory function of DMSO. On the other hand, alcohols also pointed out varying degrees of deactivation of the catalytic function of cholinesterases.

Keywords

Acetylcholinesterase, butyrylcholinesterase, Ellman's method, organic solvents.

Article History

Submitted: 30 November 2020 Accepted: 18 December 2020 Published Online: December 2020

Article Info

***Corresponding author:** Hayrettin Ozan Gulcan **email**: ozan.gulcan@emu.edu.tr

**Tugba Ercetin and Acelya Mavideniz contributed equally to this manuscript.

Research Article:

Volume: 3 Issue: 3 December 2020 Pages: 153-158

INTRODUCTION

For more than 85 years, scientists have been working on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes from various scientific perspectives. Cholinesterases are the enzymes that cleave acetylcholine to choline and acetate. These enzymes are the primary targets of drugs used in Alzheimer's disease. Moreover, they are employed as pesticides, nerve agents and drugs which are used in the treatment of myasthenia gravis and Parkinson's disease (Dingova *et al.,* 2014). Cholinesterases are also related with the etiopathogenesis of some diseases like cancer, cardiovascular diseases, and obesity (Dingova *et al.,* 2014). From this point of view, the determination of cholinesterase activity or its inhibition is crucial.

Both qualitative and quantitative assays are employed to determine the activity or inhibition of cholinesterases. These assays are applicable in many fields as well as in pharmaceutical sciences (Miao *et al.,* 2009). Cholinesterase activity and its inhibition are determined employing numerous methods (Miao *et al.,* 2009). These methods include, spectrometric (Uv-Vis (McOsker and Daniel, 1959), fluorometric (Guilbault and Kramer, 1965), diffractometric (Walker and Asher, 2005), mass spectrometric (De Jong et al., 2006) assays), thin layer chromatography (TLC) (Marston et al., 2002), radiometric (Wininteringham and Disney, 1962) and calorimetric assays (O'Farrell et al., 1977), biosensor tests (Cesarino *et al.,* 2012), colorimetric sticks or strip based assays (Augustinsson, 1957), histochemical localization of acetylcholinesterase (Koelle and Friedenwald, 1949) and chip techniques (Hadd *et al.,* 1999). Spectrometric assay is still the most extensively used technique among all of these methods (Miao *et al.,* 2009).

Ellman's method is categorized as UV-Vis spectrometric assay and it is the most prevalent type of assays which is used to determine cholinesterase activity. It provides continuous monitoring of acetylthiocholine (an alternative substrate to acetylcholine) hydrolysis by AChE under in vitro conditions (Ellman *et al.,* 1961). This assay consists of two reactions. First one is the hydrolysis of thioesters (acetylthiocholine/ATch or butyrylthiocholine/BTch) to thiocholine by cholinesterases (AChE or BChE). The second reaction involves the interaction of thiocholine with DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) which yields out a yellow product, referred to as 2- nitro-5-thiobenzoic acid (TNB) (Dingova *et al.,* 2014). Color intensity of the product that is

measured at 412 nm is proportional to the enzymatic activity (Miao *et al.*, 2010).

Ellman's method is cheap, simple, fast and accurate which makes it indispensable for the measurements and monitoring of cholinesterase activity. The activities of many cholinesterase inhibitors are measured using Ellman's method. In order to conduct these experiments, organic solvents are generally required to aid dissolution, since many compounds are not purely soluble in aqueous buffers employed.

The present study aimed to screen the effects of the widely employed organic solvents on the activity of cholinesterases. Although similar studies on the topic were conducted previously, this study provides sufficient information, since the effects of commonly used organic solvents are investigated and the human enzymes are employed.

MATERIALS AND METHODS

Organic solvents were obtained from Sigma Aldrich (CA, USA). Their purities were more than 96 % as stated on their labels. The effects of organic solvents on human AChE and human BChE were determined by modified spectrophotometric method of Ellman (1961). Human recombinant AChE (HuAChE) (Sigma) and human BChE (Sigma) were used as enzymes for cholinesterase activity studies. Acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as the substrates of the reaction. 5,5´-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. 50 mM Tris HCl buffer (pH 8.0), 6.8 mM DTNB, 2 µl of sample solutions and 10 µl of AChE/BChE solution were added. The reactions were initiated with the addition of 10 µl of acetylthiocholine iodide or butyrylthiocholine chloride. Following the incubation for 15 minutes at 27°C, the hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride was monitored by the formation of the 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines. The measurements and calculations were assessed by using SkanIt Software 2.4.5 RE for Varioskan Flash software. Percentage of inhibition of AChE and BChE was determined by the comparison of rates of reaction of the samples relative to blank sample (buffer) using the formula;

$$(E-S)/E \times 100$$

where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicates.

RESULTS AND DISCUSSION

Percent inhibitions of organic solvents, respectively at 1, 2.5, and 5% v/v concentrations are shown in Table 1 and 2.

Table 1: Effects of different organic solvents on ACHE inhibition.

% Inhibition			
Solvents	**1%˙**	**2.5%˙**	**5%˙**
Methanol	1.0 ± 0.02	3.4 ± 0.08	5.2 ± 0.11
Ethanol	1.4 ± 0.06	2.9 ± 0.17	4.8 ± 0.21
n-propanol	4.9 ± 0.13	6.8 ± 0.05	9.3 ± 0.27
iso-propanol	9.0 ± 0.07	12.7 ± 0.16	27.4 ± 0.24
n-butanol	3.8 ± 0.03	7.9 ± 0.11	9.9 ± 0.10
Ethylene glycol	0.8 ± 0.09	2.9 ± 0.11	4.4 ± 0.17
Dimethyl sulfoxide	18.2 ± 0.21	30.8 ± 0.09	41.0 ± 0.51
Acetone	9.7 ± 0.21	19.8 ± 0.24	28.9 ± 1.21
Acetonitrile	1.4 ± 0.05	2.4 ± 0.21	3.1 ± 0.25
1,4-Dioxane	17.0 ± 0.09	28.4 ± 0.17	34.5 ± 0.47
Donepezil	NT	NT	89.5± 0.68*

NT: Not tested, * 5µM concentration was employed, ˙v/v

Table 2: Effects of different organic solvents on BCHE inhibition.

% Inhibition			
Solvents	**1%˙**	**2.5%˙**	**5%˙**
Methanol	0.9 ± 0.03	1.8 ± 0.11	2.2 ± 0.07
Ethanol	0.8 ± 0.04	1.3 ± 0.08	1.5 ± 0.33
n-propanol	2.5 ± 0.08	5.2 ± 0.18	11.8 ± 0.33
iso-propanol	6.7 ± 0.10	10.9 ± 0.53	19.9 ± 0.08
n-butanol	7.5 ± 0.09	8.4 ± 0.27	10.7 ± 0.25
Ethylene glycol	1.3 ± 0.18	3.5 ± 0.08	4.9 ± 0.07
Dimethyl sulfoxide	16.8 ± 0.87	25.9 ± 1.28	44.8 ± 0.88
Acetone	6.9 ± 0.47	17.9 ± 0.88	31.4 ± 1.04
Acetonitrile	2.8 ± 0.17	5.9 ± 0.77	9.9 ± 1.21
1,4-Dioxane	10.6 ± 0.05	17.5 ± 0.22	26.9 ± 0.55
Donepezil	NT	NT	68.8± 0.14*

NT: Not tested, * 5µM concentration was employed, ˙v/v.

According to the results, varying effects of organic solvents were observed in terms of inhibition of cholinesterase enzymes. In particular, dimethyl sulfoxide (DMSO) has been found as the most potent inhibitor among the organic solvents employed. On the other hand, 1,4-Dioxane was also assessed as a potent inhibitor. Alcohols display varying degrees of inhibition. Mainly, methanol and ethanol, two of the most frequently used solvents, have shown to display weak inhibition even at 5% v/v concentrations.

In general, enzyme inhibition based assays limit the employment of organic solvents at 5%. From this perspective, DMSO, isopropanol, acetone, and 1,4-Dioxane were shown to exhibit equal or higher than 20% inhibition in AChE and BuChE catalyzed reactions. Obviously, this outcome makes the employment of these organic solvents questionable.

Indeed, organic solvents are indispensable parts of enzyme inhibition assays. Many drugs, original drug candidates and mixtures obtained through herbal extracts are not purely soluble in water. In majority of the Ellman's test, buffers are employed at the pH of 7 or 8. Although ionization depending on the functional groups at this pH might aid in aqueous dissolution, it becomes inadequate for many chemicals. Organic solvent application up to certain concentration, therefore, aids in dissolution of these compounds.

The results obviously pointed out the fact that the generalization of the limitation of organic solvent application up to 5% v/v is not suitable, since organic solvents display varying degrees of inhibition at this concentration. Ethanol, methanol, and acetonitrile appeared to be the safest organic solvents under the experimental conditions. In other words, the positive controls wherein the full activity is obtained without a specific inhibitor should include the organic solvent at the specified concentration at least in order to prevent high inhibitory potential measurements. This is particularly valid for DMSO and acetone, as they are water miscible and widely used as solubility enhancer in Ellman's method based cholinesterase inhibition assays.

CONCLUSION

In this study, the effects of some organic solvents, commonly used as solubility enhancers, on the modified Elmman's method were investigated. It was observed that each organic solvent has varying inhibitory effects. Ethanol, methanol, and acetonitrile were found to display the weakest potentials, and therefore they were evaluated as the safest solvents. On the other hand, DMSO and acetone, two of other commonly used solvents were found to have serious inhibitory potential at 5% v/v concentration. This finding definitely requires the employment of these organic solvents in full activity assays where the enzyme catalytic function is determined in the absence of an inhibitor.

REFERENCES

Augustinsson KL (1957). Assay methods for cholinesterases. *Methods Biochem Anal* **5**: 1-64.

Cesarino I, Moraes FC, Lanza MR, Machado SA (2012). Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilized on a composite of polyaniline-carbon nanotubes. *Food Chem* **135**(3):873-879.

De Jong CF, Derks RJE, Bruyneel B, Niessen W, Irth H (2006). High-performance liquid chromatography- mass spectrometry-based acetylcholinesterase assay for the screening of inhibitors in natural extracts. *J Chromatogr A* **1112**(1-2): 303-310.

Dingova D, Leroy J, Check A, Garaj V, Krejci E, Hrabovska A (2014). Optimal detection of cholinesterase activity in biological samples: Modifications to the standard Ellman's assay. *Anal Biochem* **462**: 67-75.

Ellman GL, Courtney D, Andres V, Jr., Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7**: 88-95.

Guilbault GG, Kramer DN (1965). Resorufin butyrate and indoxyl acetate as fluorogenic substrates for cholinesterase. *Anal Chem* **37**: 120-123.

Hadd AG, Jacobson SC, Ramsey JM (1999). Microfluidic assays of acetylcholinesterase inhibitors. *Anal Chem* **71**(22): 5206-5212.

Koelle GB, Friedenwald JS (1949). A histochemical method for localizing cholinesterase activity. *Am J Ophthalmol* **70**(4): 617-622.

Marston A, Kissling J, Hostettmann K (2002). A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. *Phytochem Anal* **13**(1): 51-54.

McOsker DE, Daniel LJ (1959). A colorimetric micro method for the determination of cholinesterase. *Arch Biochem Biophys* **79**: 1-7.

Miao Y, He N, Zhu JJ (2010). History and new developments of assays for cholinesterase activity and inhibition. *Chem Rev* **110**: 5216-5234.

O'Farrell HK, Chattopadhyay SK, Brown HD (1977). Calorimetry of cholinesterase activity in serum. *Clin Chem* **23**(10): 1853-1856.

Walker JP, Asher SA (2005). Acetylcholinesterase-based organophosphate nerve agent sensing photonic crystal. *Anal Chem* **77**: 1596-1600.

Wininteringham FPW, Disney RW (1962). Radiometric assay of acetylcholinesterase. *Nature* **195**: 1303.