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Magnetic Resonance Assays of Haloperidol in Human Serum Albumin

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Abstract

Haloperidol is a commonly used neuroleptic drug associated with a range of side effects. The aim of the study was to determine *in vitro* interaction of haloperidol with human serum albumin (HSA) by ¹⁹F Magnetic Resonance Spectroscopy (MRS) at 9.4 Tesla (T). The practical measurement based on fluorine resonance has been proposed to determine drug level in HSA at 37°C.

Keywords: ¹⁹F Magnetic Resonance Spectroscopy; haloperidol; human serum albumin.

Abbreviations used: ¹⁹F, fluorine-19; MRS, Magnetic Resonance Spectroscopy; HSA, Human Serum Albumin.

Introduction

Human serum albumin (HSA) is a protein found in body fluids and blood plasma which binds to drugs and can have a significant impact on their pharmacokinetics (Petitpas I *et al.*,

2001). Moreover, HSA occurs in human body as a single polypeptide chain of 585 amino acids with weight 66.5 kDa (Dugaiczky A *et al.*, 1982). The protein binds to several water insoluble endogenous compounds such as unesterified fatty acids (Curry S *et al.*, 1988), bilirubin (He XM and Carter DC., 1992), bile acids (Kragh-Hansen U, 1990) and thus facilitates their transport throughout circulation in the body. Interaction between the HSA and drug causes increase of blood plasma carrying capacity for insoluble molecules and often decreases its toxicity (Sudlow G *et al.*, 1975). Therefore, the study of neuroleptic drugs currently is an emerging area of preclinical research..

Most of the recent studies have been using analytical assays to map out the number of binding sites on the HSA (Dockal M *et al.*, 2000). This approach uses a variety of the spectroscopic methods such as Ultra Violet (UV) (Sakurai T and Tsuchiya S, 2006), circular dichroism (CD) (Wilting J *et al.*, 1980), fluorescence (Sudlow G *et al.*, 1976) as well as electron spin resonance (Hsia JC *et al.*, 1978).

In order to study the binding of drug to HSA we examined the effect with haloperidol upon the relaxation of the fluorine atom. Haloperidol, 4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidyl]-1-(4-fluorophenyl)-butan-1-one, has one fluorine atom in the structure to be attached to the aromatic ring (Figure 1). The already reported methods for the determination of haloperidol in body fluids involve the following techniques: reaction with 1,2-naphthoquinone-4-sulphonic acid (Darwish IA, 2005), fluorimetry (Baeyens, 1977), polarography (Ozkan SA *et al.*, 2003), proton (^1H) magnetic resonance spectroscopy (Bustillo JR, 2008), spectrophotometry (Askal H.F (1997), high performance liquid chromatography (HPLC) (Walter S *et al.*, 1998) and gas chromatography with mass spectrometry (GC-MS) (Ulrich S *et al.*, 1998). Recently, the method for determination of haloperidol in human serum was developed while using HPLC with fluorescence detection. Suzuki coupling reaction with a fluorescent arylboronic acid, 4-(4,5-diphenyl-1H-imidazol-2-yl)phenylboronic acid (DPA), was employed to convert haloperidol into highly fluorescent compounds (Kishikawa N *et al.*, 2006). However, the use of Magnetic

Resonance (MR) technique has advantage because it enables the direct study of haloperidol without prior treatment.

Current analysis of haloperidol indicates that a limit may exist for haloperidol efficacy; values above this limit seem not to provide any supplementary clinical improvement and may even reduce therapeutic effect (Ulrich S *et al.*, 1998). It has been clinically documented that haloperidol causes often extrapyramidal side effects (motor disturbances) including acute dystonic reactions, acathisia syndrom, drug-induced Parkinsonism, bradykinesia, akinesia, tardive dyskinesia symptoms associated with neurotoxic properties of neuroleptic drugs and malignant syndrome (Cesario V, 2008). The plasma elimination of haloperidol in humans is much slower with time after dosing, and near terminal elimination half-life may be measured in days rather than hours (Hubbard JW *et al.*, 1987). Side effects such as parkinsonian symptoms and neuroleptic malignant syndrome may persist for weeks after discontinuation of neuroleptic drugs (Kornhuber J and Weller M, 1994). Therefore, after discontinuation of haloperidol, their side effects are still present for a prolonged period. It is not known whether the extended effects of haloperidol in humans are due to the continued presence of drug in brain tissue or to long-lasting drug-induced physiologic changes (Cohen BM *et al.*, 1992). However, haloperidol interactions with HSA are still poorly understood.

In this work, we applied Fluorine-19 Magnetic Resonance Spectroscopy (^{19}F MRS) at 9.4 Tesla (T) MR system as a tool for identification and quantification of haloperidol. The application of non-proton MRS to *in vitro* and *in vivo* monitoring of drug has the advantage over standard ^1H MRS. ^1H and ^{13}C are nuclei which are most often exploited in MR resonance investigations of biomolecules and drugs. However, ^1H and ^{13}C MRS of biomolecules and drugs are usually complicated and not easy for interpretation due to many proton-proton or carbon-carbon couplings. Of particular interest is ^{19}F that has MR sensitivity similar to ^1H . 100% natural abundance of ^{19}F and its high MR sensitivity (81% of ^1H nucleus), make ^{19}F MR a reliable analytical tool for the study of organofluorine compounds. ^{19}F MRS is only restricted to the monitoring of the bio-transformation of ^{19}F containing drugs, as there is no endogenic fluorine compound in living organisms.

To date, the only one study on haloperidol (Shamsipur M *et al.*, 2007) was the study with the application of ^{19}F MRS to assay haloperidol in tablets and haloperidol dissolved in HSA with deuterium solution (D_2O) transferred into *in vitro* samples as an internal frequency lock at 11.7 T. *In vitro* ^{19}F MR has been also already used to aid pharmacodynamics and pharmacokinetics of several medically important fluorinated drugs including antineoplastic fluoropyrimidines (Martino R *et al.*, 2006). The application of ^1H NMR has been limited because of the large molecular mass of HSA causing signal broadening (Kitamura K *et al.*, 2007). A wide range of chemical shift (300 ppm) of ^{19}F compounds ensures good separation of signals in different environments. Furthermore an internal reference enables quantification without the need for the separation or derivatization (Ellis DA *et al.*, 2000). Importantly, such ^{19}F MRS studies translate well into clinical setting as allows non-invasive monitoring of drug metabolism and pharmacokinetics *in vivo* (Malet-Martino M *et al.*, 2006). Taking these aspects together it is important to determine haloperidol blood levels. The use of ^{19}F MRS in our study was aimed at investigating the interaction of haloperidol with HSA.

Material and methods

Materials

Haloperidol, trifluoroacetic acid (TFA) and formic acid were of analytical grade and supplied by Sigma-Aldrich (Oakville, ON). Human serum albumin (HSA) was obtained from Biofluids (Oakville, ON).

Preparation of in vitro samples

5 mg of standard haloperidol was used to prepare stock solution in 10 mL of water. Formic acid was added dropwise to achieve $\text{pH} = 3.5$ and 60 mg/mL concentration of HSA was prepared in phosphate buffered saline (0.05 M, $\text{pH} 6.8$). Haloperidol solution was dissolved in an appropriate amount of HSA to give final concentration of 1.5, 5, 30, 60, 120, 180, 240, 300, 360, 420 and 800 $\mu\text{g/mL}$ of haloperidol. The samples were incubated at 37°C for 2 hours in order to reach binding equilibrium.

All ¹⁹F MRS measurements were performed using a 9.4T magnet with bore diameter of 21 cm (MagneX, UK) and TMX console (NRC-IBD, Canada) operating at 376 MHz. Spectra were recorded at a physiological temperature of 37°C using 5-mm solenoidal probe (NRC-IBD). Approximately 10 mL of the sample solution was transferred in an MR glass tube. The ¹⁹F signal of trifluoroacetic acid (TFA) with constant concentration in water equal to 1 mg/mL was used as an internal reference for ¹⁹F chemical shift. The signal of TFA was calibrated for chemical shift $\delta = 0.0$ ppm. The measured integration of ¹⁹F signal of haloperidol was compared to integration of TFA signal at each haloperidol concentration used. For all samples ¹⁹F MRS was performed using one pulse sequence (flip angle 60°; time to repetition (TR) = 800 ms; number of average (NA) = 2 and time to echo (TE) = 6 ms). The signal to noises ratio was equal three. The areas of the fluorine resonances in the spectra were analyzed using MAREVISI software (NRC-IBD, Canada). The quantitative measurements were made by measuring ratio of peak areas. Having established the concentration of TFA, the concentration of haloperidol in HSA solution were established from observed ¹⁹F signals. The number of repetitions to reproduce the results was $n = 6$ for each haloperidol concentration.

The accuracy of the ¹⁹F MRS measurements of drug concentration was assessed using two samples with 240 and 420 µg/mL of haloperidol concentration. The starting concentration for both samples was 60 µg/mL of haloperidol. Then the drug was added to obtain the final concentration of 240 and 420 µg/mL, respectively.

The time-stability of the measurements of drug concentration was determined daily by utilizing the coefficient of variation (CV, %) in day first and day third for 1.5, 5, 30, 60, 120, 180, 240, 300, 360, 420 and 800 µg/mL concentrations of haloperidol.

Analysis of haloperidol binding

Haloperidol binding to HSA has been studied at pH 7.4 and 37°C. The binding equilibrium of drug can be summarized as described in Eq. 1: $H + HSA \rightleftharpoons H - HSA$ (Eq. 1).

where: H – haloperidol; HSA - human serum albumin; $H - HSA$ complex of haloperidol and human serum albumin.

The constant equilibrium is given as Eq. 2: $K = \frac{[H - HSA]}{[H][HSA]} = \frac{[H - HSA]}{[H][HSA]_0 - [H - HSA]}$ (Eq. 2).

where: $[H]$ - concentration of haloperidol; $[HSA]$ - concentration of human serum albumin; $[H - HSA]$ - concentration of the complex of haloperidol and human serum albumin; $[HSA]_0$ - initial concentration of human serum albumin.

The fraction of bound haloperidol (p_b) is given by: $p_b = \frac{[H - HSA]}{[H] + [HSA]_0} \approx \frac{[H - HSA]}{[H]}$ (Eq. 3) where

$[H]$ and $[H - HSA]$ were calculated from integration of fluorine signals in relation to peak area of the TFA.

Results

Haloperidol solutions alone and solved in HSA were used to obtain spectra at high field MR. Haloperidol itself showed a single signal of its fluorine atom at -29 (δ , ppm). The chemical shift of ^{19}F acquired in haloperidol at concentration of 1.5, 5, 30, 60, 120, 180, 240, 300, 360, 420 and 800 $\mu\text{g/mL}$ in HSA solution was -9 ± 0.5 (δ , ppm). TFA was selected as an internal standard for spectra due to its sharp ^{19}F singlet from CF_3^- and was calibrated at 0.0 (δ , ppm). Upon the addition of haloperidol solution to HSA, the measured single ^{19}F signal shifted and broadened. Moreover, the occurred single signal of ^{19}F in HSA solution indicated that HSA has one binding site for haloperidol, thus the drug together with HSA form a 1:1 complex at each used haloperidol concentrations.

We calculated the integral of haloperidol and TFA peaks and took their ratio. Our observations suggest that the detection limit of ^{19}F of haloperidol was 1.5 $\mu\text{g/mL}$. The linear regression was used to analyze the data and the correlation coefficient between analytical concentrations take to reaction and measured concentrations on collected ^{19}F spectra was found to be $R^2 = 0.92$. ^{19}F MRS procedure shows to be rapid and selective. The accuracy (in 240 and 420 $\mu\text{g/mL}$ concentration of

haloperidol with starting concentration 60 µg/mL) was 91-95%. The time stability of haloperidol concentration in HSA, as determined by the value of the coefficient of variation in day first and day third for each sample was consistently within 4-7%. The fraction of bound of the haloperidol to HSA (p_b) was 0.89 ± 0.2 . The acquired data are presented as mean $\pm$ SD. The calculation was done using Sigma Stat Pro (Chicago, IL).

Discussion

One of the novel and most important applications of high field MR in pharmaceutical sciences is its ability to examine protein-drug interactions (Skinner J and Laurence JS, 2008). The MRS parameters of a ligand, such as chemical shifts, generally change on binding to a protein, and there is often difficulty in using ^1H MRS spectroscopy to study protein–ligand binding directly because of the need to extrapolate the observed small molecules signals to obtain their values in the bound states to large protein. The problems that may occur when using ^1H MRS to study small molecules in aqueous protein solutions is chemical shift overlap of protein and ligand resonances and the broadening of the ^1H MRS resonances of the small ligand caused by rapid relaxation. Alternatively, in this study we have demonstrated the ^{19}F MRS measurements which monitor the changes of small molecule during interaction with HSA. ^{19}F MR has been already used in clinical studies of mapping fluorine drugs in human brain (Lyo IK and Renshaw PF, 2002). The result of work in this area is the fact that accumulation of agents in the brain can be much slower than in plasma (Mason GF and Krystal JH, 2006). In addition, drug level in brain can exceed drug level in plasma (Strauss WL and Dager SR, 2001). Therefore, it is important to provide rapid method to determine plasma level of neuroleptic drugs (Cohen *et al.*, 1980).

In studying the interaction of drugs and proteins, fluorescence techniques are commonly used because of their high sensitivity, rapidity and ease of implementation. Several reports have been published studying the interaction of proteins with drugs by fluorescence technique (Liu *et al.*, 2004). However, the multiple binding sites for anesthetics in human serum albumin (HSA) make solution studies difficult to interpret. The method with UV detection loses sensitivity when extracts

of samples are analyzed because of interference caused by the UV-absorbing of many co-extracted compounds. Degradation of the components can also occur in analyzed samples due to the long extraction process.

The most suitable compounds for the measurements of ^{19}F MRS are those containing multiple chemically equivalent fluorine atoms, such as in trifluoromethyl (CF_3) groups, due to their high NMR signal per molecule. To date detection of monofluorinated paroxetine in the brain (Henry E *et al.*, 2000) was with level of 6 $\mu\text{g/mL}$. Also the concentrations of the trifluoperazine (Komoroski RA *et al.*, 1991) and fluphenazine (Durst P *et al.*, 1990) were detectable by ^{19}F MRS in brain at levels 4 -5 $\mu\text{g/mL}$.

In our *in vitro* study, it was possible to observe ^{19}F signal in the HSA with detection limit of 1.5 $\mu\text{g/mL}$. The starting recommended dose of haloperidol for patients is 10 mg/day and the maximum dose is 30 mg/day (Goldman Z *et al.*, 1981). Moreover, in human blood plasma with a HSA concentration of about 50–60 mg/mL, haloperidol is approximately 90% bound to the plasma proteins (Kudo S and Ishizaki T, 1999).

Recent studies suggest that haloperidol provides rapid therapeutic plasma levels and also can play a role in treating psychiatric emergencies. However, the mean (percent coefficient of variation) haloperidol bioavailability (over 15 minutes) in plasma after 2.5 mg (2.5 mg/0.1-ml spray) intranasal administration was 63.8 % and 48.6 % for 2.5 mg (5.0 mg/ml) intramuscular administration (Miller JL *et al.*, 2008).

Various analytical techniques available to determine drugs concentration in plasma are too time consuming for routine clinical use. The ^{19}F MRS study shows that the spectroscopy offers quantitative determination of haloperidol in water solution. However, changes in fluorine chemical shifts can be observed when using D_2O for ^{19}F MR spectroscopy. The proton-deuterium exchange can influence the fluorine chemical shift (Oldfield E *et al.*, 1980). Therefore, ^{19}F MRS in an ordinary water solution offers conditions close to that encountered in the real plasma sample. The presented *in vitro* ^{19}F MRS method is suitable for measurements of haloperidol in HSA with

quantitative accuracy and can be applied to study other fluorine containing drugs. It can be possible that the presented measurement will find the use in the determination of drug pharmacokinetics study or in drug design studies, where binding needs to be optimized.

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Figure Captures

Fig. 1. The structure of haloperidol.