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"*In vitro*" MRI findings of an agent can be used in the imaging of GABA receptors and similar agent's future projections

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Abstract. – OBJECTIVE: The goal of this study was to analyze magnetic resonance imaging findings of gamma-amino butyric acid and gamma deuteroxy sodium butyrate and to evaluate possible "*in vitro*" and "*in vivo*" areas of use.

MATERIALS AND METHODS: Materials used included gamma-amino butyric acid and gamma deuteroxy sodium butyrate which is formed by the replacement of the deuteroxy group. An amino group in the gamma position of gamma-amino butyric acid was evaluated with a standard magnetic resonance device with the power of 1.5 Tesla and a brain coil. These findings have been compared.

RESULTS: Gamma deuteroxy sodium butyrate has shown to have statistically different signals than gamma-amino butyric acid, 0.09 NaCl, distilled water and gadolinium chelates.

CONCLUSIONS: Findings suggest that "*in vivo*" studies should be conducted in addition to phantom studies; Deuterium imaging may be used with or without proton imaging with technical support. Other agents may be studied for "*in vivo*" use following the labeling with Deuterium.

Key Words:
Gamma deuteroxy sodium butyrate, GABA, Deuterium, MRI, Contrast agents, Molecular imaging.

Introduction

Contrast agents that are used in magnetic resonance imaging (MRI) are: superparamagnetic iron oxide particles and dissoluble paramagnetic metal complexes (Fe^{2+}, Fe^{3+}, Mn^{2+}, Gd^{3+} or Dy^{3+}). These materials provide contrast formation based on local magnetic field changes. Because of the different structures of these materials, there are different specific and general areas of use for them. Especially Gadolinium (Gd) which has been used in MRIs since 1988 because of its high paramagnetic characteristics and long relaxation duration. However, its use has been limited because of the adverse effect risks which have become pronounced in recent years[1-3].

In recent years, development of functional and anatomic detail imaging (fMRI, BOLD, molecular imaging) with the use of endogen materials or tissue characteristics is in process of physiological environment and pathologic processes[4,5].

Selective replacement of Hydrogen with Deuterium (^2H) (deuteriumation) creates a potential for the development of new drugs from physiologically active components and although it was given little attention until recently, it presents an area of possible use as a powerful chemical medical mediator.

There is a long history of deuteriumated components used in humans as metabolic and pharmacokinetic probes. Deuteriumated components have been evaluated widely in non-clinical enviroments and have been used in humans as metabolic or pharmacokinetic probes[6].

However, Deuterium because of its different "gyromagnetic ratio", has a different Larmor frequency than ^1H. Therefore, it does not form signals (images) in ^1H sensitive standard MR devices.

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter of the brain and is found throughtout the central nervous system (CNS). GABA is synthesized and released in the endings of GABAergic neurons.

The first step of researching these experimental drugs was to analyze the findings of standard MRIs of gamma deuteroxy sodium butyrate (GDSB) which is formed by the replacement of the deuteroxy group with the amino group in gamma position of GABA. Currently GABA agonist drugs on the market are used in the hydroxy form (XYREM®), and to evaluate GABA agonist agents as possible "*in vitro*" and "*in vivo*" areas of use.

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Materials and Methods

Chemicals, Pharmaceuticals and Reagents

Gamma-butyrolactone was purchased from Sigma-Aldrich Chemical, Seelze, Germany. Sodium deuterium oxide 30% in deuterium oxide, potassium bromide, sodium chloride, pure ethanol, and pure water were purchased from Merck Chemical, Darmstadt, Germany. Dotarem was produced by from Guerbet Pharmaceutical, France. Gadovist and Magnevist were purchased from Bayer Schering Pharmaceutical, Berlin, Germany. Multihance was purchased from Bracco Pharmaceutical, Milan, Italy. Omniscan was purchased from Amersham Health, Cork, Ireland.

Synthesis of γ-deuteroxysodiumbutyrate (GDSB)

Eighteen ml of γ-butyrolactone was added to a 25 ml 30% solution of sodium deuterium oxide in deuterium oxide. This solution was boiled under a reflux condenser for three hours. At the end of this time 100 ml of pure water was added to dissolve the salt and the solution was filtered and evaporated under reduced pressure. The salt was recrystallized from pure ethanol.

Measurements of IR Spectra

The obtained product was confirmed with a IR spectrophotometer (Figure 1). The infrared transmission spectra were produced by a PerkinElmer Spectrum One spectrometer (Melville, NY, USA). Measurements were carried out using the KBr method. A KBr disk without any sample and a KBr optical plate were used as references in the KBr method. The measurements were conducted in a room used exclusively for IR spectral measurements. The humidity and temperature were controlled to 30-40% and 23°C, respectively. KBr disks and pastes were usually prepared in the controlled room, but occasionally in an ordinary laboratory.

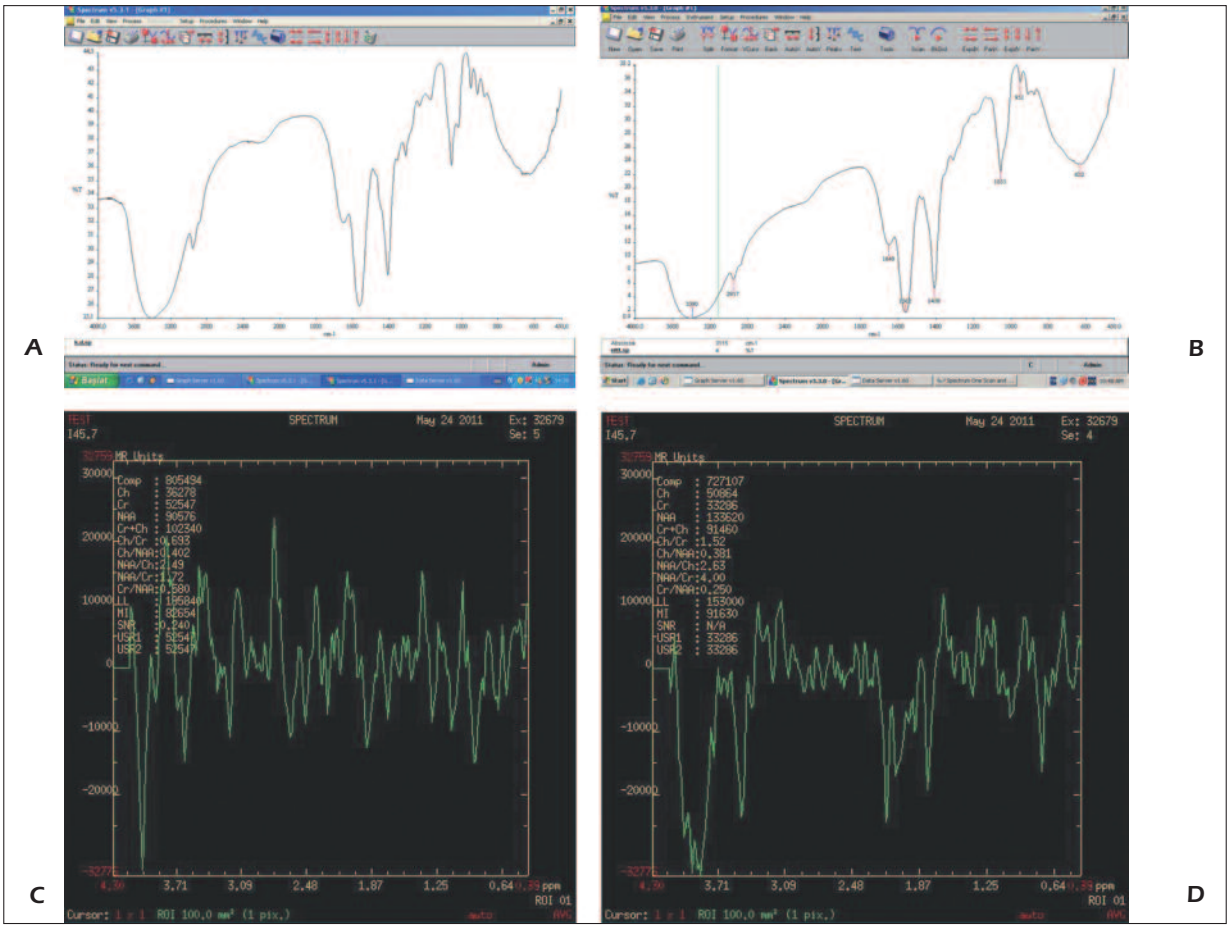

Figure 1. IR SpectrometrI ***A-B,*** and MRS ***C-D,*** of GABA and GDSB pre post process, respectively showed similarity of depression of signals.

MRI Modeling

The "*phantom*" was formed as tubes were placed 3 × 3 in a supor made of poplar wood (12 × 12 cm, with 9 holes of 3 cm in diameter) (Figure 2a). The tubes were plastic centrifuge tubes of 50 ml volume and 3 cm diameter (Figure 2b).

GDSB and GABA were poured into tubes. The dose of 1.67 mg/ml would be achieved in the total body fluid volume of distribution (distribution volume as ≈600 ml/kg).

Other commonly available MR contrast agents; Dotarem®, Gadovist®, Magnevist®, MultiHance® and Omniscan™ were also poured into tubes. The dose of 0.1 mg/ml would be achieved in the extracellular fluid volume of distribution (distribution volume as ≈200 ml/kg).

One tube of pure distilled water, one tube of 0.9% NaCl and an empty (air) tube were prepared as well.

All tubes were placed into the phantom as shown in Figures 2a and 3a.

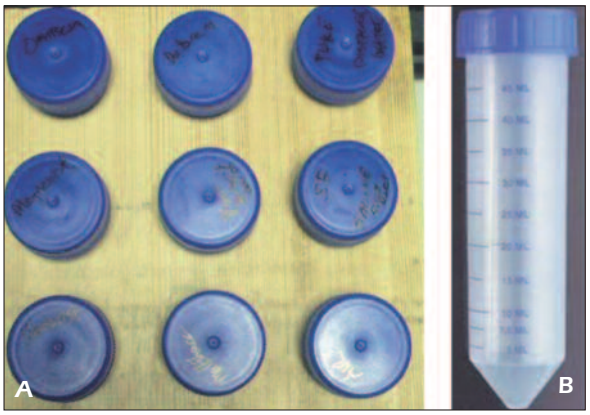

Figure 2. Tubes support ***(A)*** and plastic tube ***(B)*** for using phantom.

Imaging and Evaluation of the Images

Imaging was conducted with a MR device with the power of 1.5 Tesla (1.5T GE Signa HDxt scanner, General Electric Healthcare, Waukesha, USA)

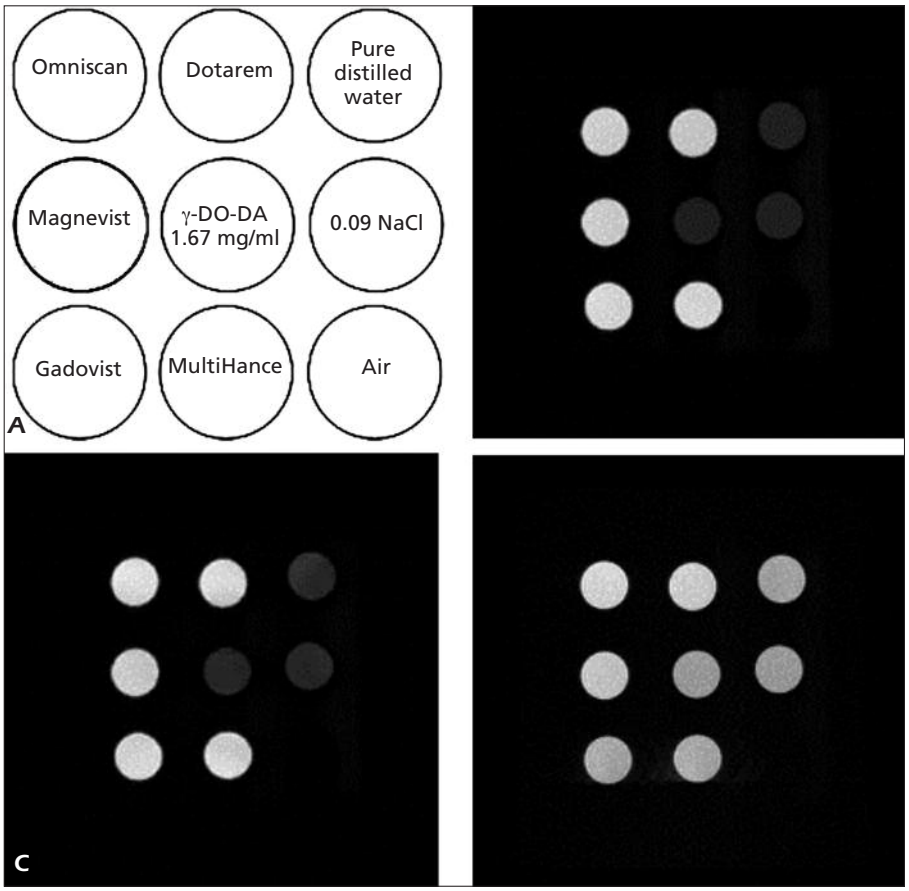

Figure 3. Arrangement ***(A)***, T1 weighted image ***(B)***, T1FS weighted image ***(C)*** and T2 weighted image ***(D)*** of tubes containing of Gd-chelats, Pure Distiled Water, Air and GDSB, so was that like used instead of GABA with GDBS.

and brain coil (8-channel HD Brain Coil). The parameters of the MRI scanning were the field of view: 240 mm; slice thickness 7 mm and Spin echo. Matrix, Repetition Time (TR) and Echo Time (TE) values for T1 weighted, T1 weighted fat saturation and T2 weighted were 256 × 256, 566.668, 8.896; 256 × 256, 1000, 8.896; 512 × 512, 2133.34, 82.68 respectively.

The images were evaluated with a Free DICOM viewer (Onis 2.3 Free Edition, Digitalcore, Tokyo, Japan).

Values were automatically recorded for each tube with a constant ROI in each sequence (number, mean, standard deviation). SNR and CNR values were calculated.

Statistical Analysis

The p value was calculated with a Statistic Calculator Version 8.0 (StatPac Inc, Minneapolis, MN, USA) using an "independent groups *t*-test between means" test. $p < 0.05$ was considered statistically significant.

Results

GDSB formed a significantly lower signal in T1 ($p < 0.001$) and a significantly higher signal in T1FS and T2 ($p < 0.001$) than GABA (Figures 4, 5 and 6). Because of this, Deuterium was replaced with Hydrogen and it decreased the GDSB's MR signals.

Also, the distilled water and 0.9% NaCl formed similar signal values. Despite the similarity of the values, the values of distilled water was found significantly higher in T1, T1FS and T2 ($p < 0.001$). This sample contained a higher amount of hydrogen.

As expected, it was observed that GD chelates formed signals in the form of close value cumulating.

In the T1 weighted images, GDSB and GABA groups formed signals which were 9.22 times lower than the Gd chelates ($p < 0.001$) and 1.93 times lower than the distilled water-0.9% NaCl group ($p < 0.001$).

In the T1FS weighted images, GDSB and GABA groups formed signals which were 7.47 times lower than the Gd chelates ($p < 0.001$) and 2.07 times lower than the distilled water-0.9% NaCl group ($p < 0.001$).

In the T2 weighted images, GDSB and GABA groups formed signals which were 2.38 times lower than the Gd chelates ($p < 0.001$) and 2.06 times lower than the distilled water -0.9% NaCl group ($p < 0.001$).

These findings showed that the deuteriumated agent had different signal because of its reduced hydrogen content.

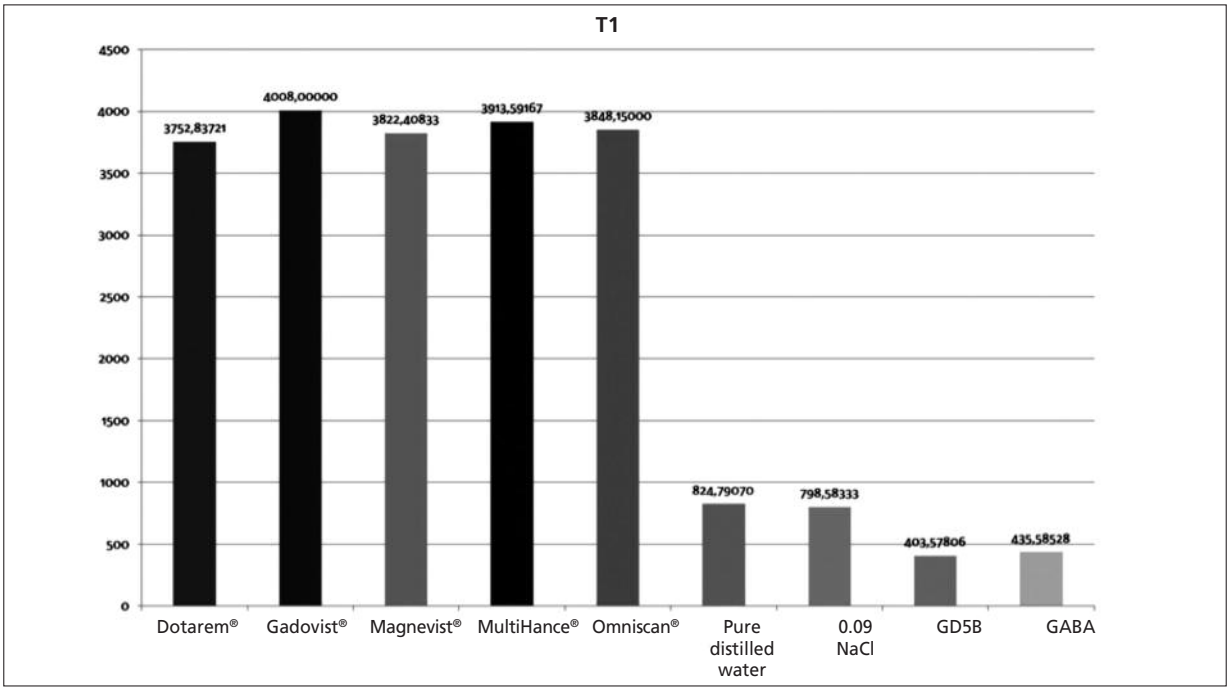

Figure 4. T1 weighted signal intensity mean values of agents.

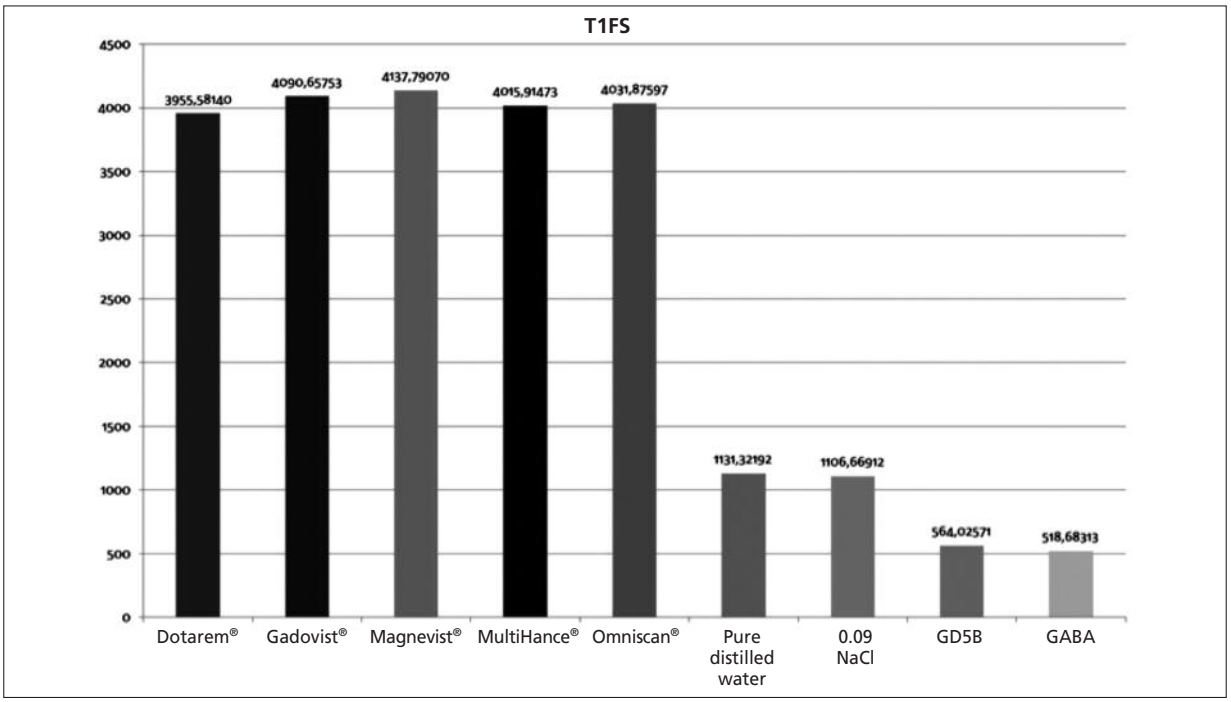

Figure 5. T1FS weighted signal intensity mean values of agents.

Discussion

Several key criteria have to be satisfied to image specific "*in vivo*" molecules. These are usability as a probe with a high affinity with suitable pharmacodynamics. The ability of these probes to overcome biologic intercommunication obstacles such as: vascular, interstitial, and cell

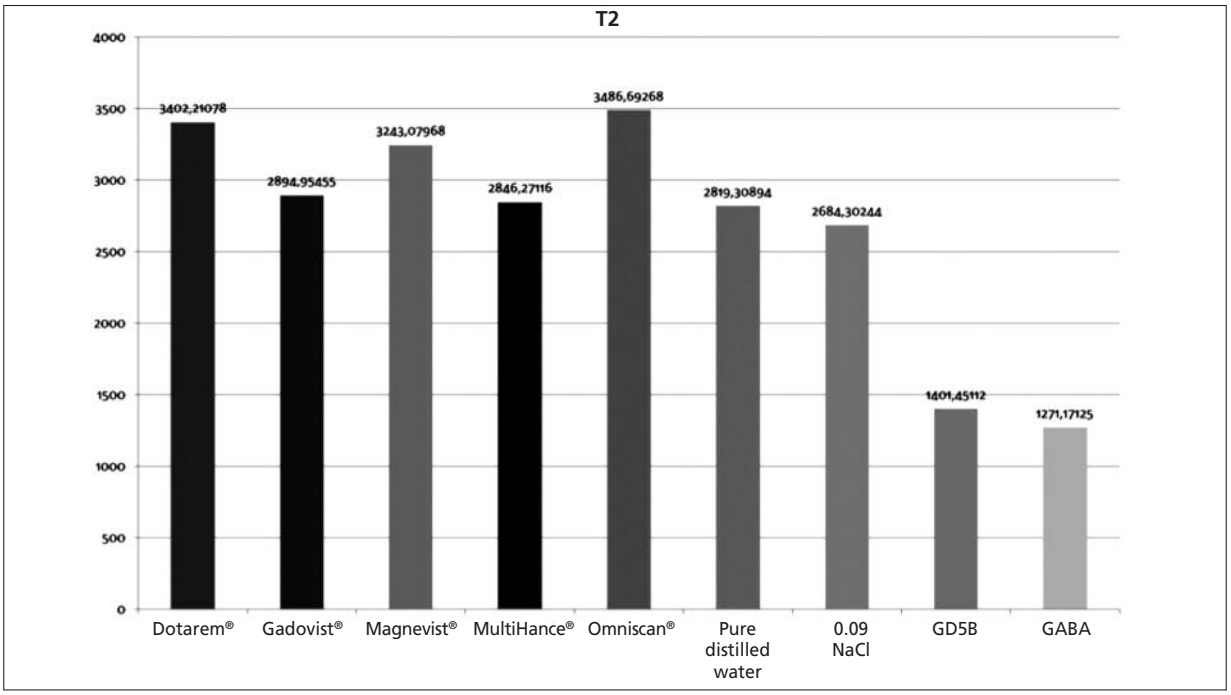

Figure 6. T2 weighted signal intensity mean values of agents.

membranes. Use of amplification strategies (chemical and biologic) and usability of imaging techniques which are sensitive, rapid and with a high resolution. For a successful "*in vivo*" imaging on a molecular level, all four pre-conditions should be fulfilled[7].

GABA is an important inhibitor neurotransmitter in the adult mammary brain. GABA is synthesized and released in the endings of GABAergic neurons. It plays a role in the regulation of neuronal excitability in all nervous systems[8,9].

It was observed that GABA functions with the trigger of binding GABA to its ionotropic [GABA (A) and GABA (C)] and metabotropic receptors [GABA (B)] in ligand-gated chloride channels. Variability of pharmacologic properties of the subtypes of GABA receptors is clinically important[8,9].

GABA is in clinical use because of its high tolerability and it has a high affinity probe ability and has a sufficient volume of distribution in CNS[9].

Deuterium is a natural stable hydrogen isotope in D_2O which constitutes 0.015% of water in nature[10].

Deuterium has been used as tracer/probe in nutrition physiology and biochemistry studies regularly. In addition to healthy adult volunteers, athletes, also pregnant, lactating women, children and infants less than 1 year old were included in these studies[11].

It has been shown that acute replacement of 15-23% of total body plasma with deuterium did not cause any significant adverse effects. In routine studies, a maximum 500 mg/kg D_2O has been administered to infants and babies less than 3 months old[11-13].

This dose which is accepted as safe for humans including infants and children is much higher than the recommended doses of deuteriumated GABA agonist.

It has already been theorized that replacement of 10% of body fluids (approximately 50 mg/kg) with D_2O has no significant health effects. Large amounts of animal data adjusted for humans has shown that the risk of D_2O related side effects of deuteriumated GABA agonist was only a remote possibility[11].

Deuteriumation with the components which pharmacologic effects on humans are well defined may fill the unmet needs of certain patients and may provide an opportunity to create new patented drugs[6].

When deuterium is replaced with hydrogen molecules, the deuteriumated components resemble hydrogenated components in almost all features. Since electron clouds of combined atoms define the shape of the molecules, the shape and dimensions of deuteriumated components cannot be differentiated from their totally hydrogenated analogs[6,14].

If they are close to the ionizable groups, there are minor physical changes in some features such as lowered hydrophobia or changed pKa. These may be determined in partially or totally deuteriumated components compared to their totally hydrogenated analogs[6].

These differences are small enough to detect any changes in the selectivity of pharmacologic targets related to the deuteriumation of a non-covalent drug or its biochemical effect capabilities[6].

To evaluate the "*in vivo*" biochemical efficacy of a physiologically active component, more studies are needed.

Deuterium (^2H), because of its different "gyromagnetic ratio", has a different Larmor frequency than ^1H [^1H: 42.577 (kg^{-1}.s.A), ^2H: 6.535 (kg^{-1}.s.A)]. When Hydrogen is replaced by Deuterium, its peak in the infrared spectrum shifts because of the Fermi resonance effect. Its molecular vibration stops or diminishes. Therefore, it does not form signals (images) in currently used ^1H sensitive standard MR devices. Special hardware and software modifications are needed.

The study showed, a deuteriumated agent (GDSB) in physiologic doses formed significantly different signals than GABA, water, physiologic serum and gadolinium chelates in T1W, T1FS and T2W sequences.

Conclusions

These findings suggest that "*in vivo*" studies should be conducted in addition to phantom studies; deuterium imaging may be used with or without proton imaging (it is especially useable in other than T1W sequences), with technical support (hardware-software), with "merge" or "subtraction" for MRIs. Other agents such as glucose may be studied for "*in vivo*" use following the labeling with deuterium.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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