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A Multi-Sample Dissolution DNP System for Serial Injections in Small Animals

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Abstract

Purpose

A number of experimental in-vivo applications of dissolution Dynamic Nuclear Polarization (DNP) requires rapid successive injections of hyperpolarized substrates. Here we present the design and performance of a home-built multi-sample dissolution DNP setup for small animal research.

Methods

The DNP setup consists of a commercial wide-bore magnet charged to 3.35 T, a cryostat, a 94 GHz microwave source and a custom-built skeleton, which accommodates four identical sample sticks. Each sample stick features a dissolver locked into the skeleton port and a lifter, which permits moving the sample cup out of the liquid helium bath for dissolution.

Results

The dissolution of the first sample was triggered after 2 hours of polarization build up during single-shot operation of the cryostat. Thereafter a time window of 75-90 min was available to dissolve the remaining three polarized samples. The average liquid state-polarization over all four sticks was measured as $18.7 \pm 2.3\%$ for $[1-^{13}\text{C}]$ pyruvate at 30 sec after dissolution. In-vivo applicability of the setup using serial injections of $[1-^{13}\text{C}]$ pyruvate to study cardiac metabolism in rats revealed good reproducibility.

Conclusion

The simple four-sample DNP insert described here provides reproducible liquid state polarization of $[1-^{13}\text{C}]$ pyruvate and allows for rapid repeat injections in small animals.

Key words

Dynamic nuclear polarization, dissolution DNP, multi-sample DNP, metabolic imaging, small animal

Introduction

Dissolution Dynamic Nuclear Polarization (DNP) (1) has gained considerable attention as it provides >10'000-fold signal enhancement of relevant molecules for MR imaging and spectroscopy studies. In DNP, highly polarized electron spins are used to increase the polarization of nuclei of interest in a low-temperature environment using microwave irradiation (2). The transfer of polarization requires between 30 min and 3 hours depending on various parameters such as the static magnetic field strength, the radicals used or the type of nuclei involved. After polarizing the nuclei, samples are rapidly dissolved using pressurized, heated water and the final solution is then available for in-vivo studies (1). With reasonably sized molecules, T_1 relaxation times in liquid state are sufficiently long to enable MR imaging of the turnover of metabolically active substrates (3-7). To separate different metabolic products from the injected substrate, chemical-shift selective imaging or decoding is exploited providing unparalleled insights into in-vivo metabolism in real time (8-11). The method is promising for both oncology (12) and cardiovascular applications (13). Recent work has also demonstrated the feasibility and safety of the concept in humans provided that sterile compounding is possible (14).

A key strength of dissolution DNP using endogenous substrates is the repeatability of injections thereby providing access to metabolic information at various time points during disease progression and therapy. To study transient metabolic changes during pharmacological or structural interventions, however, consecutive injections in rapid succession are required. For example, shortage of oxygen supply to the heart leads to insufficient production of high-energy phosphates and impairment of contractile function within minutes (15).

Different single-sample DNP polarizers have been described in the literature (1,16-19) following similar concepts. The original single-sample, integrated cryostat design by Ardenkjaer-Larsen et al. (1) has been further developed and commercialized (HyperSense, Oxford Instruments, UK). While the combined magnet/cryo design of the HyperSense device has advantages, it also complicates maintenance. To this end, home-built polarizers have primarily been constructed using standard vertical-bore NMR magnets with field strengths between 3.35 and 7 Tesla and with separate cryostats (16-22). Dedicated home-built inserts are used to implement the DNP functionality including capabilities to assess NMR and ESR signals for diagnostic purposes.

In recent work the potential of cross-polarization to shorten the long polarization build-up times in dissolution DNP has been highlighted (23,24). In addition, application of an additional modulation of the microwave irradiation field proved to reduce the build-up time of ^1H nuclei in experiments conducted with nitroxide radicals (25). Nevertheless, sample loading and polarization times remain relatively long compared to requirements of many interventional in-vivo studies. Given the long time constants of polarization build-up and hence the inability to provide hyperpolarized samples for rapid repeat metabolic studies, devices for concurrent polarization of multiple samples have been proposed (20,21,26). While the commercial four-sample sterile device by Ardenkjaer-Larsen et al. (26) is designed to provide large sample volumes for potential human applications, its purchasing and running costs may not be justified for small-animal imaging experiments.

The objective of the present work is to describe the design and performance of a low-cost four-sample dissolution DNP insert for standard wide-bore NMR magnets and cryostats providing hyperpolarized liquid-state samples with a minimum latency of 3 min. The

applicability of the device is demonstrated using sequential dissolutions and imaging of cardiac metabolism in rats at four different time points within one hour.

Methods

Beside the DNP insert, the general setup presented here is similar to our previous multi-sample polarizer (20). In brief, a standard NMR cryostat (Oxford Instruments, UK) is mounted in a shielded 7 T wide-bore (89 mm) magnet (Bruker Biospin, Switzerland) charged to 3.35 T. The DNP insert operates with a 94 GHz microwave source (Elva, Russia). A generic data acquisition system with multi-purpose interface (National Instruments, USA) is used in conjunction with home-built LabView software (National Instruments, USA) to control the system.

Cryogenic system

The cryogenic system includes a variable-temperature insert, a liquid-helium transfer line, and vacuum pumps. Large-diameter tubing (ISO-KF 40) is used to minimize the pressure drop along the exhaust line. A roots-pump (Okta 500, Pfeiffer Vacuum, Switzerland) in series with a dual-stage rotary-vane pump (Duo65, Pfeiffer Vacuum, Switzerland) is employed to pump on the helium. To fill the system, liquid helium is drawn through the transfer line from a liquid-helium supply dewar. A needle valve is used to control the liquid helium flow into the bottom of the cryostat and the sample space. A helium recovery system is connected to recycle helium gas and to reduce the overall running costs.

To prepare the system for operation, the cryostat is pumped with the exhaust gas guided through a tube surrounding the helium-supply capillary within the transfer line, thereby precooling helium. To achieve temperatures below 3 K, the cryostat is operated in single-shot

mode after filling. In this case helium is pumped directly through an exhaust port on the cryostat. The liquid-helium level is monitored using a capacitive sensor built from two stainless steel tubes. Temperature sensors (Cernox, USA) are mounted in the cryostat vacuum shield at the bottom of the helium bath and on the copper baffle above the sample space.

DNP insert

A custom-built skeleton, which can accommodate up to four identical sample sticks for concurrent polarization, is inserted into the cryostat (Figure 1). The maximum sample volume per stick is 135 μl . Each sample stick features a dissolver locked into the skeleton port upon loading and a lifter which permits moving the sample cup by approximately 5 cm longitudinally for polarization/dissolution (Figure 2). For polarization the sample is in the lower position within the liquid helium bath and lifted above the liquid-helium level for dissolution. The lifter is operated using a mechanical pulling rod. Upon pulling the lifter, the sample holder docks into the dissolver forming a chamber separated from the cryostat vacuum.

Microwave system

Microwaves are guided from the source by a short piece of WR10 waveguide coupled to a circulator followed by a tapered transition to an oversized WR28 waveguide. In the skeleton the waveguide is fed centrally between the sample sticks to the magnet center using an E-bend and a transition from copper to stainless steel including a mylar window as a vacuum seal. The internal flanges are custom-made due to space limitations. No dedicated microwave cavity or horn antenna is used. To confine the microwave energy to some extent in the sample space, the cryostat walls and lowest skeleton baffles are made from copper.

NMR diagnostics

For NMR signal monitoring a single loop “B” shaped coil was mounted between the sticks in the symmetry plane of the waveguide and connected to a 3.5 mm, 50 Ohm semi-rigid transmission line consisting of stainless steel and copper (upper part) / beryllium copper (lower part) to reduce thermal conductivity into the cryo space.

Operation

An experiment cycle starts with sample-stick loading at 10-20 K cryostat temperature, filling and cooling the cryostat with liquid helium. Thereafter, the helium inlet is closed and the pumps are operated at maximum power while directly connected to the cryostat. Using microwave irradiation, all four samples are polarized simultaneously. The sample sticks are lifted and dissolved independently from each other without pressurizing the cryostat. For dissolution a selected stick is connected to the dissolution device, the water heater is filled with dissolution buffer (Tris), pressurized and heated to 170°C. Subsequently, the sample is moved up by 44 mm to pull it above the liquid-helium level (Figure 2) and the dissolution is triggered. During this process microwaves are temporarily switched off. The valve on the collector opens automatically and the sample fluid is collected into a syringe. At the end of an experiment cycle the liquid-helium flow is restarted, the cryostat pressurized to retract the empty sample sticks and a new cycle can be started.

In-vivo measurements

Four healthy female Sprague Dawley rats (250-300g) were anaesthetized using 1-2% isoflurane in an air/oxygen (4:1) mixture, endotracheally intubated and placed in a Bruker Biospec 9.4T small animal MR system. Body temperature was kept at 37-38 °C. The animals were mechanically ventilated at 60 bpm and heart rate was continuously monitored (300-400 bpm). To enhance bicarbonate signal, the animals received an iv glucose infusion (Bichsel AG, Interlaken, Switzerland; 15 mg glucose per kg of body weight per minute). All animal experiments were performed in adherence to the Swiss law of Animal Protection and approved by the Zurich cantonal veterinary office. A birdcage dual $^1\text{H}/^{13}\text{C}$ coil (Rapid Biomedical, Wurzburg, Germany) was used for transmission, and a ^{13}C surface coil from the same vendor was used for signal reception. Linear order shimming was performed by minimizing the water line width using a localized spectroscopic pulse sequence with a voxel covering the left ventricle. The shimming sequence was triggered to respiratory and cardiac motion. Samples containing 50.8 μl of [1- ^{13}C] pyruvic acid, 13.5 mM trityl radical and 1 mM Gadolinium (Dotarem, Guerbet, France) were polarized and dissolved using 8 ml of 50 mM Tris/NaCl buffer (pH 7.5). The liquid was collected in a collector containing 650 μl of 1 M NaOH. Rats were injected 1 ml of the final solution of 44 mM [1- ^{13}C] pyruvate. A spatial-spectral excitation pulse was used in combination with a multi-echo EPI readout (number of echoes 7, echo time 5.04 ms, echo time increment 0.383 ms, FOV 60x40 mm², matrix 48x22, partial Fourier factor 0.69, in-plane resolution 1.25x1.25 mm², slice thickness 5 mm) to dynamically image the conversion of pyruvate into lactate and bicarbonate with a repetition interval of 1.5 s during a total scan duration of 5 min (27). Metabolic images were reconstructed using the IDEAL approach (28) resolving pyruvate, lactate, bicarbonate, pyruvate hydrate and alanine resonances. Using the spatial-spectral excitation pulse, the nominal flip angles on the pyruvate, lactate and bicarbonate resonances were 4, 19 and 19

deg, respectively. Four repeat injections were performed in each animal at 0, 20, 40 and 60 min to demonstrate rapid sequential dissolutions. Signal intensity time curves of lactate and bicarbonate were integrated to obtain the area-under-the-curve (AUC). Lactate-to-bicarbonate AUC ratios were calculated in four myocardial segments to assess in-vivo reproducibility.

Results

System performance

Optimum microwave output power at the source was 40 mW. The temperature near the sample was ≤ 1.35 K during single-shot mode. Upon dissolution of an individual sample, the temperature increased to about 2 K but returned back to the initial value within 2-10 min depending on the remaining helium level (Figure 3). The helium consumption during single-shot operation was approximately 115 ml/h. The heat load introduced by the dissolution of the four sticks consumed an additional amount of 180 ml of helium. Given the helium level prior to the dissolution of the first sample, a time window of 75-90 min was available to dissolve the remaining three samples. The black line of the NMR signal build-up curve shown in Figure 3 is corrected for differences in coil loading and sample volume associated with successive dissolution/removal of samples. Liquid state-polarization for the four sticks was measured from a total of 24 experiments as $18.7 \pm 2.3\%$ at $t = 30$ sec after dissolution and extrapolated back to $t = 0$ sec resulting in polarization values of $36.4 \pm 4.5\%$ for measured T_1 values of 45.2 ± 4.0 sec at 9.4T. Individual polarization levels of dissolved $[1-^{13}\text{C}]$ pyruvate for sticks #1-4 are given in Figure 4. The order of dissolution of stick #1-4 was randomized during 5-7 runs per stick.

The total time required for one experiment cycle includes sample loading (5 min), pre-cooling (15 min), filling of the cryostat (40 min), switch to single-shot mode (10 min), DNP polarization buildup (90-120 min), sample dissolutions, stick retrieval and reestablishing of helium transfer (5-10 min). Accordingly, the first sample is available for dissolution after 160 min. The dissolution of the last sample should happen within 90 min after the first dissolution given the helium consumption in single-shot mode. The minimum interval between successive dissolutions is currently 3 min, which is given by refilling, switching and connecting the single

water heater to different sticks. A volume of 8 ml of the dissolution medium was found to give reproducible dissolution results.

In-vivo measurements

Example metabolic maps of pyruvate, lactate and bicarbonate of the heart and corresponding signal intensity time curves obtained sequentially in one animal are shown in Figure 5. In the left-ventricular blood pool, peak pyruvate SNR across all four animals was measured to be 41.9 ± 26.7 (mean $\pm$ SD), while lactate and bicarbonate SNR in the myocardial segments were 9.9 ± 3.3 and 8.9 ± 2.5 , respectively. The lactate-to-bicarbonate AUC ratio over all animals and dissolutions was 1.22 ± 0.23 . The difference in lactate-to-bicarbonate AUC ratio between injections was $2.3 \pm 15.7\%$, 0.3 ± 16.8 , $0.7 \pm 8.8\%$ and $0.7 \pm 13.2\%$ for anterior, septal, inferior and lateral myocardial segments, respectively. Intra-subject reproducibility of the lactate-to-bicarbonate ratio was assessed using the relative mean absolute deviation resulting in 6.1%, 8.8%, 3.0% and 10.8% for the four individual animals, respectively. There was no trend seen in the lactate-to-bicarbonate ratio as a function of injection across this small population.

Discussion

A home-built, multi-sample dissolution DNP insert has been presented permitting dissolution of up to four hyperpolarized samples within a 75-90 min time window with a minimum interval of 3 min between consecutive dissolutions. In-vivo, sequential dissolution and metabolic imaging of pyruvate metabolism in the rat heart with a time interval of 20 min has been demonstrated with an overall good intra-subject reproducibility of lactate-to-bicarbonate ratios in four myocardial segments across four animals. The interval of 20 min between injections was chosen to reduce stress to the animals.

Operation of the dissolution DNP system is facilitated by the fact that all components remain in the cryostat under vacuum and switching of microwave and dissolution paths as required with other systems is not necessary (1,16-19,21). Compared to other multi-sample systems published elsewhere (29) the dissolution volume could be restricted to 8 ml hence minimizing dead volumes during prospective in-vivo experiments in small animals. Further volume reduction to 6 ml was tested and is possible. Alternatively, inert fluids with subsequent separation could be considered (30) to reduce the amount of substrate.

Liquid-state polarization levels and T_1 time constants of $[1-^{13}\text{C}]$ pyruvate obtained in this work are in line with literature values. To increase polarization levels beyond those obtained here, the system can straightforwardly be ramped to twice the field (6.7 T). Given the low microwave power required at 3.35 T (40 mW), a microwave frequency multiplier can be used in conjunction with the current source, which provides a maximum power of 200 mW. In addition, further improvement in liquid state polarization available at the time of injection could be obtained by reducing the sample transfer time with a dedicated shuttling system including magnetic shelter tunnel and/or automatic injection setup (31,32). Liquid-state polarization levels and T_1 time constants of $[1-^{13}\text{C}]$ pyruvate obtained in this work are in line

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The solid-state NMR signal reported in Figure 3 was corrected for intrinsic signal contributions from the sample holder and lifter material as well as for differences in coil loading and sample volume depending on different combinations of sample stick states (polarization/dissolution). To address any inaccuracies in this correction step, separate NMR coils for each stick need to be accommodated.

The duration of one experiment cycle is currently limited by the helium consumption in single-shot mode. To address this limitation an additional helium reservoir can be inserted into the cryostat to allow for rapid helium refilling or continuous flow operation (19). Our current insert geometry was designed with this aspect in mind and can accommodate an additional helium reservoir in the future.

The material cost, besides the magnet price, of the presented system is approximately 80'000 US\$ and includes the cost for the vacuum pumps, microwave source, NMR console, cryostat, transfer line, DNP insert components and NMR diagnostics. The cost of the NMR magnet adds another 100'000 US\$. To keep the cost of operation low, the system was connected to our institutional helium recovery system.

Conclusion

In this work a simple four-sample DNP insert suitable for standard NMR magnets and helium-temperature cryostat components was constructed and demonstrated to provide reproducible liquid state polarization of [1-¹³C] pyruvate for rapid repeat injections in small animals.

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Figure legends

Figure 1. Schematic of DNP setup (a) including wide-bore magnet (1), helium dewar (2), vacuum pumps (3), microwave source (4), cryostat (5), DNP insert skeleton (6), sample collector (7), water heater (8), dissolution valve/connector (9), sample space (10), sample sticks (11). Insert containing one sample stick (b) and close-up (c) with waveguide (12), heat baffles (13), helium level sensor (14), NMR coil (15), and sample cup (16).

Figure 2. Details of sample stick configuration during polarization and dissolution. Stick tube (1), lifter in the upper position (2), inlet tube (3), outlet tube (4), dissolution coupling (5), sample cup during dissolution (6), liquid helium level (7) and sample cup during polarization (8).

Figure 3. Microwave activity, temperature, helium level* and NMR signal during polarization/dissolution of stick #1-4 as a function of time ($t=0$ refers to start of single-shot mode). Intermittent temperature increase upon dissolution is rapidly equilibrated. Helium consumption in single-shot mode was approximately 115 ml/h. The NMR signal is corrected for differences in coil loading and sample volume (black curve). (* the helium level sensor has a limited range and hence a decrease in helium level is only seen after 60 min as indicted by the dashed box).

Figure 4. Polarization and T_1 time constants (mean $\pm$ SD) of [1- 13 C] pyruvate samples of sticks #1-4 at $t=30$ sec after dissolution. Stick numbers refer to physical sticks. The order of dissolution and position during 5-7 runs per stick was randomized.

Figure 5. In-vivo imaging example. Maps of [1- 13 C] pyruvate, [1- 13 C] lactate and bicarbonate as well as signal intensity time curves acquired at 0, 20, 40 and 60 min in the same animal demonstrate good reproducibility.

Figures

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