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SHORTENING OF NMR 1/T_1 AND 1/T_2 RELAXATION RATE DISTRIBUTION INTERVALS IN D_2O CONTAINING JAW CYSTS OR ABSCESSES: SEPARATION OF CYSTS FROM ABSCESSES[**]

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It is well known that the relaxation rates (1/T_1 and 1/T_2) in H_2O/D_2O mixtures are smaller than those in H_2O alone. The results of this have not been examined in the presence of a low rate of cysts or abscess contents in D_2O. One purpose of this study was to determine the shortening of the relaxation rate distribution interval (SRDI) for D_2O containing cysts or abscess contents. Another goal is to investigate the differentiation of cysts from abscesses with T_1 and T_2 measurements. The ultimate objective is the possibility of a new MRI contrast mechanism for dental lesions. Nineteen odontogenic jaw cysts and 12 jaw abscesses were collected from patients. A mixture was prepared by adding 0.06 mL cyst or abscess content to 0.94 mL D_2O. The experiments were carried out with a nuclear magnetic resonance spectrometer operating at 400 MHz. The mean 1/T_2 of the abscesses was significantly different from that of the cysts ($P < 0.005$), but the mean 1/T_1 of the non-haemorrhagic cysts was not different from that of the abscesses ($P = 0.626$). The interval shortening ratios (SRDIs) of 1/T_1 and 1/T_2 for the cystic mixture were 16.36 and 4.22, respectively. The ratios were 8.39 and 2.68, respectively, for the mixture containing abscess contents. In conclusion, the relaxation rate reduction of the mixture results in a shortening of the distribution intervals, which leads to the separation of cysts from abscesses. In addition, the high interval shortening ratios indicate that the results of this study can provide a basis for studies on a new MR contrast mechanism for jaw lesions.

Keywords: *Jaw cysts, Jaw abscesses, D_2O solutions, NMR T_1 and T_2, relaxation distribution interval.*

СОКРАЩЕНИЕ ИНТЕРВАЛОВ РАСПРЕДЕЛЕНИЯ СКОРОСТЕЙ РЕЛАКСАЦИИ ЯМР 1/T_1 И 1/T_2 В D_2O-СОДЕРЖАЩИХ КИСТАХ ИЛИ АБСЦЕССАХ ЧЕЛЮСТИ: ОТДЕЛЕНИЕ КИСТ ОТ АБСЦЕССОВ

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Проведены исследования дифференциации кист и абсцессов с помощью сокращения интервала распределения скорости релаксации (SRDI) 1/T_1 и 1/T_2 для D_2O-содержащих кист или содержимого абсцесса с целью возможности создания нового механизма контрастирования МРТ для поражений зубов. У пациентов собрано 19 одонтогенных кист челюсти и 12 абсцессов челюсти. Смесь готовили путем добавления 0.06 мл содержимого кисты или абсцесса к 0.94 мл D_2O. Эксперименты проводились на ЯМР-спектрометре, работающем на частоте 400 МГц. Среднее значение 1/T_2 абсцессов значительно отличалось от такового для кист ($P <0.005$), но среднее значение 1/T_1 негеморрагических кист не отличалось от такового для абсцессов ($P = 0.626$). Коэффициенты SRDI 1/T_1 и 1/T_2 для кистозной смеси 16.36 и 4.22, для смеси с содержимым абсцесса 8.39 и 2.68, т. е. снижение скорости релаксации смеси приводит к сокращению интервалов распределения, что способствует отделению кист от абсцессов. Высокие коэффициенты сокращения интервалов указывают, что полу-

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ченные результаты могут стать основой для исследований нового механизма магнитно-резонансного контрастирования при поражениях челюсти.

Ключевые слова: *киста челюсти, абсцесс челюсти, раствор D_2O, T_1 и T_2 ЯМР, интервал распределения релаксации.*

Introduction. Magnetic resonance imaging (MRI) has often been shown to distinguish jaw cysts from other jaw lesions such as abscesses, granulomas, ameloblastomas, and odontogenic tumors [1–3]. Despite such studies, magnetic resonance imaging in cysts and abscesses is still of interest for clinical practice and scientific research.

D_2O has been used as a solvent since the discovery of NMR. The proton spin-lattice relaxation rate ($1/T_1$) for degassed water is approximately 0.3/s for H_2O and 0.03/s for D_2O [4]. Such a decrease in the D_2O relaxation results in a shortening of the relaxation distribution interval of D_2O solutions containing body fluids. This may reduce the overlap between the relaxation rates of D_2O containing different fluids and may increase the likelihood of the separation of different fluids.

In D_2O solutions, including those containing cysts or abscesses, most of the normal hydrogen atoms are replaced with deuterium, and HOD molecules are formed [4–7]. Therefore, the $1/T_1$ and spin-spin relaxation rate ($1/T_2$) in D_2O solutions are shorter than those in H_2O solutions. Accordingly, compared with natural samples, mixtures of 94% D_2O and 6% cysts or abscesses provide smaller relaxation rates and shorter relaxation distribution intervals. In addition, $1/T_1$ and $1/T_2$ relaxation rates in cysts and abscesses are related to their viscosity [8]. Dilutions of small amounts of cysts and abscess contents in D_2O minimizes the effects of the viscosity and contributes to the shortening of the relaxation rate distribution interval (SRDI) [9]. Due to this shortening, the overlap between the relaxation rates of D_2O containing cyst and abscess contents can be decreased, and the possibility of separating cysts from abscesses can be increased. In addition, an infusion of D_2O into tissue, blood flow and other heterogeneous systems has been proposed as a new MRI contrast mechanism [10–13]. Therefore, this study may provide a basis for future MRI investigations for diagnostic purposes.

The first purpose of this study is to obtain shorter relaxation rate distribution intervals for D_2O solutions containing cysts or abscess contents. Another goal is the segregation of cysts from abscesses using relaxation measurements. The possibility of a new contrast mechanism for dental lesions is the ultimate objective. For this purpose, 0.06 mL of sample (cyst or abscess) was added to 0.94 mL of D_2O (99.9%). Relaxation measurements were performed at 400 MHz. Groups were compared statistically.

Materials and methods. *Sample collection, storage, and preparation.* Sample collection and storage were essentially the same as in previous studies [8, 14]. The contents of 19 odontogenic jaw cysts and 12 jaw abscesses were collected from patients of the Oral-Maxillofacial Surgery Department. Each sample was centrifuged at 2500*g* for 5 min for red cell separation, transferred to a tube with a cap and then sealed using parafilm. All sample-containing tubes were placed in plastic containers with lids, sealed again with parafilm, and then stored at –5°C in a refrigerator. Six of the 19 cysts contained high amounts of blood, and the red cells of these cysts were not separated by centrifugation. These samples were considered haemorrhagic cysts. The clinical evaluations for the diagnosis of cysts and abscesses were confirmed by radiological and histological studies.

The sample in each capped tube was dissolved in the NMR laboratory prior to the relaxation measurements. After shaking each tube, a mixture was prepared by adding 0.06 mL of sample (cyst or abscess) to 0.94 mL of D_2O. The mixture was carefully shaken before each measurement. Before and after each relaxation measurement, the homogeneity of each mixture was checked and found to be stable. D_2O including 99.9% D atoms was purchased from Merck KGaA (Sigma Aldrich).

Relaxation measurements. The experiments were carried out with a Bruker Avance III NMR spectrometer operating at 400 MHz. The spin-lattice (T_1) and spin-spin (T_2) relaxation times of the water in the samples were measured using the water signal detected at 4.71 ppm (Fig. 1). The T_1 measurements were performed by the inversion recovery method. Delay times between 180 and 90^o pulses were altered from 0.1 to 30 s (Fig. 2). The pulse repetition time was set at 60 s to allow full recovery of the magnetization in the mixtures. T_2 measurements were carried out by the Carr-Purcell-Meiboom-Gill method. In this case, the echo delays varied from 40 ms to 3 s (Fig. 3). The magnetization recovery curve for T_1 and the magnetization decay curve for T_2 were a single exponential each. The sample temperature was set to 22 ± 0.10^{o}C by using a temperature-controller unit. The experimental errors were estimated approximately 2% for T_1 and 3% for T_2.

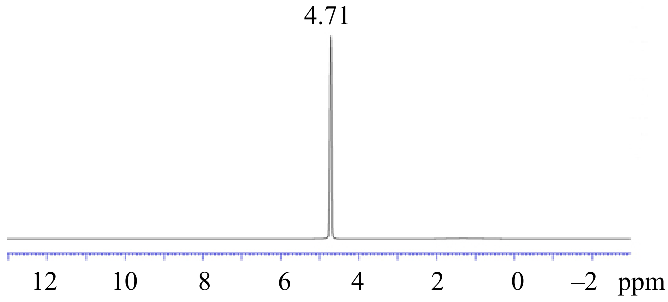

Fig. 1. Proton NMR spectrum for a mixture containing 94% D_2O and 6% cysts.

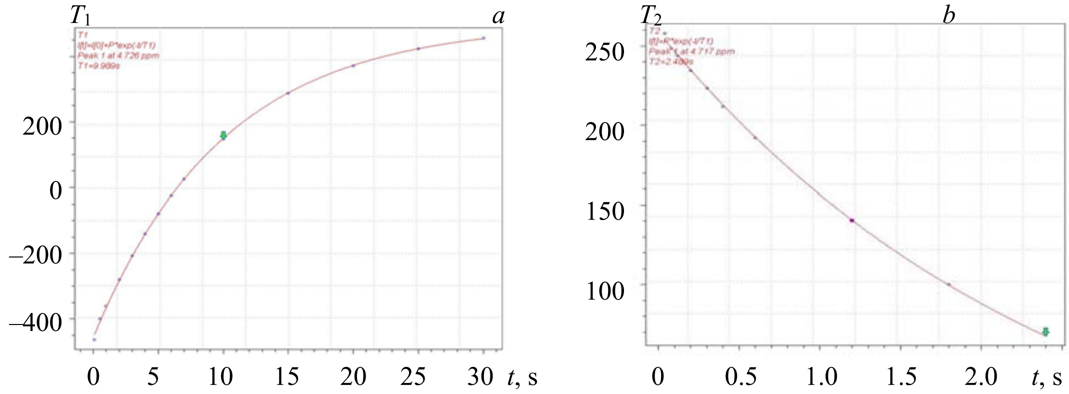

Fig. 2. Measurement of the spin-lattice relaxation time using (a) the inversion recovery method (T_1) and (b) the Carr-Purcell-Meiboom-Gill method (T_2).

Grouping of samples and statistical evaluation. Samples were grouped as odontogenic cysts, haemorrhagic cysts, and abscesses. The T_1 and T_2 values measured for each group were converted to $1/T_1$ and $1/T_2$. The results of statistical analysis are presented as the means ± standard deviations (SDs). One-way analysis of variance (ANOVA) and Tukey's HSD post hoc tests ($\alpha = 0.05$) with 95% confidence were used for multiple comparisons. $P < 0.05$ was considered statistically significant.

Maximum relaxation rate distribution interval and shortening of relaxation rate distribution interval. To increase the reliability of the evaluations, the difference between the maximum and minimum relaxation rate values obtained for the mixture was considered the maximum relaxation rate distribution interval (MRDI). The division of the SD (found for the relaxation rates of natural cysts and abscesses) to the MRDI was taken as the SRDI for the mixture.

Results and discussion. The mean $1/T_1$ and $1/T_2$ values of the mixtures together with SD and MRDI for both $1/T_1$ and $1/T_2$ are given in Table 1. The current SDs for each group are quite small. The SD and MRDI found for $1/T_2$ are much larger than those obtained for $1/T_1$. The SD and MRDI values in Table 1 are much smaller than the previous SD values given for natural cysts and abscesses [14].

Comparisons between the mean values of the groups are given in Table 2. The P values in Table 2 are smaller than those previously obtained for natural cysts and abscesses [14].

Despite the decrease in the SD and MRDI, the mean $1/T_1$ of the odontogenic cysts was not different from that of the abscesses ($P = 0.626$). However, the mean $1/T_1$ of the haemorrhagic cysts was different from that of the odontogenic cysts ($P = 0.001$). In addition, the mean $1/T_2$ of the abscesses was completely different from that of the odontogenic ($P < 0.001$) and haemorrhagic cysts ($P = 0.005$).

The highly significant P values in Table 2 indicate that the cysts in the mixtures can be discriminated from abscesses with the T_2 measurements. Similarly, the T_1 measurements can differentiate haemorrhagic cysts from other cysts and abscesses. The significant P values in Table 2 correspond to the reduced overlap between the $1/T_1$ or $1/T_2$ values of the groups. The present results are consistent with previous studies revealing the diagnostic value of MRI for jaw lesions and abscesses [15–17]. The results are also consistent with T_2 studies that demonstrated the differentiation of cysts and hepatic malignancies from abscesses and other lesions [14–18].

TABLE 1. Mean $1/T_1$ and $1/T_2$ Values Measured from D_2O Solutions Containing 6% Cyst or Abscess

Group	$1/T_1$ ± SD (1/s)	$1/T_2$ ± SD (1/s)	MRDI for $1/T_1$ (1/s)	MRDI for $1/T_2$ (1/s)
Odontogenic cysts	0.099 ± 0.015	0.579 ± 0.071	0.032	0.291
Haemorrhagic cysts	0.129 ± 0.010	1.845 ± 0.264	0.025	0.579
Abscesses	0.103 ± 0.010	1.493 ± 0.149	0.032	0.510

N o t e. Standard deviations (SDs) and the maximum relaxation rate distribution intervals (MRDIs) are also presented.

TABLE 2. Comparisons between Mean Relaxation Rates Measured in the Mixtures (6% cyst or abscess and 94% D_2O)

Comparisons between	*P* values for $1/T_1$ comparisons	*P* values for $1/T_2$ comparisons
Odontogenic cysts and abscesses	0.626	0.001
Haemorrhagic cysts and abscesses	0.001	0.005
Odontogenic cysts and haemorrhagic	0.001	0.001

The MRDIs calculated in this study were compared with the SDs obtained for the natural samples in our previous study [14]. The results are given in Table 3. The relaxation distribution intervals (SRDIs) for the $1/T_1$ and $1/T_2$ of cystic D_2O are approximately 16 and 4 times shorter than those of natural cysts, respectively. Meanwhile, the SRDI values for the $1/T_1$ and $1/T_2$ of D_2O with abscesses are nearly eight and three times shorter than those of natural abscesses.

TABLE 3. The Ratios Calculated for the Shortening of the $1/T_1$ and $1/T_2$ Distribution Intervals (SRDIs) in the Mixtures (6% cyst or abscess and 94% D_2O)

	Natural samples SD ($1/T_1$)	mixture MRDI ($1/T_1$)	SRDI in $1/T_1$	natural samples SD ($1/T_2$)	mixture MRDI ($1/T_2$)	SRDI in $1/T_2$
Cyst	0.5893	0.036	16.36	1.2291	0.291	4.22
Abscess	0.2851	0.034	8.39	1.3679	0.510	2.68

N o t e. Data given for natural samples (cysts and abscess) were taken from our previous paper [14].

In our previous study, the mean $1/T_1$ and $1/T_2$ in natural cysts were found to be 0.9355/s and 2.4575/s, respectively. The mean rates in natural abscesses were 0.8245/s and 4.7073/s, respectively [14]. The comparison of these values with the mean $1/T_1$ and $1/T_2$ values of the mixtures given in Table 1 clearly explains the reason for the interval shortening. The mean $1/T_1$ and $1/T_2$ obtained for the mixtures containing cysts (not SRDIs) are 9.5 and 4.2 times shorter than those of natural cysts, respectively. The mean $1/T_1$ and $1/T_2$ for the mixtures containing abscesses are, respectively, 8 and 3.2 times shorter than those of natural abscesses. The shorter $1/T_1$ and $1/T_2$ values in the mixture with respect to those of the natural samples are consistent with both the SRDI values and those in D_2O [4, 19]. Such shorter $1/T_1$ and $1/T_2$ result from weak H-D interactions in D_2O solutions of cysts, abscesses, and proteins [4–7, 19]. Accordingly, the SRDI ratios of the groups are related to the shorter $1/T_1$ and $1/T_2$ values obtained in the mixtures. Furthermore, the $1/T_1$ and $1/T_2$ values in cysts and abscesses depend on their viscosity and solid content [9, 14, 20, 21]. Therefore, dilution of the cyst and abscess with D_2O minimizes the effects of viscosity and solid content and contributes to the shortening of the interval. As a result, the high *P* values given in Table 2 are related directly to the shortening of the relaxation distribution intervals, which results in less overlap.

The frequency dependence of H_2O and D_2O solutions containing protein or enzyme has been described in various studies [4, 19]. The ratio of the H_2O data to the D_2O data in Fig.1 of [19] (nearly 0.365/0.052) is 7.02 at approximately 60 MHz or 1.5 T (between 10^7 and 10^8 rad/s) for $1/T_1$. Similar results can be obtained using the T_1 data from Fig. 1 of [4]. The data in these figures are consistent with our SRDI results presented in Table 3. This indicates that our results are also valid in terms of the MR frequency. In addition, D_2O has been suggested as a contrast agent for hydrogen MR [10–13]. Since heavy water (D_2O) can be easily applied to dental cysts and abscesses, MRI can be used to separate cysts from abscesses and other lesions. It is well known that MR facilities are available in hospitals, and dental MR machines are used even for routine purposes [22]. Furthermore, low-field NMR machines are quite cheaper than high-field machines. Therefore, low-field NMR can be easily established in any surgical department. In other words, the physical infrastructure for the implementation of existing results is available in most hospitals.

The contrast in MRI is based on the $1/T_1$ and $1/T_2$ relaxation rates, which depend on the sample composition [1–3, 8, 14–18, 20]. The components of samples containing concrete components cause strong H-H interactions in H_2O and weak H-D interactions in D_2O [4, 19]. In the current study, we used weak H-D interactions to separate cysts from abscesses [4, 5, 19]. These interactions correspond to the respective physical states of all sample components and contribute to the prevailing relaxation mechanisms [4, 19]. Therefore, the differentiation of cysts from abscesses is directly related to the physical state (weak H-D interaction) of the sample components, including concrete constituents. This approach can also be used to diagnose other pathological fluids or lesions.

It is well known that in vitro NMR studies lay the groundwork for in vivo MR studies and routine clinical MR. Since the application of D_2O isotonic saline solution to jaw lesions is a direct procedure, it should be more efficient than intravenous administration. The above literature and our data strongly suggest that the method used in this study can be adapted for routine dental MR.

Conclusions. The separability of T_1 and T_2 has been greatly improved for certain groups by the use of D_2O as the solvent. D_2O causes shortening of the relaxation distribution intervals, which in turn causes less overlap between the relaxation rates of some groups. This leads to the separation of cysts from abscesses. This study should provide a basis for future investigations on diagnostic MRI studies.

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