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Evaluation of Hydration Effect on Human Skin by ^{1}H MRS at 14.1T

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Abstract : Purpose : We achieved high resolution MR imaging and spectra of human skin *in vitro* with using a 14.1 T MRI/MRS system, and evaluated the hydration effect of various cosmetic products by measuring the skin's moisture concentration. Materials and Methods : We used the Bruker 14.1 T MRI/MRS system with a vertical standard bore that was equipped with a DMX spectrometer gradient system (200 G/cm at a maximum 40 A), RF resonators (2, 5 and 10 mm) and Para Vision software. Spin echo and fast spin echo pulse sequences were employed for obtaining the high resolution MR images. The 3D-localized point resolved spectroscopy (PRESS) method was used to acquire the MR spectra. Results : The high resolution MR images and spectra of human skin *in vitro* were successfully obtained on a 14.1T system. The water concentration of human skin after applying a moisturizer was higher than that before applying a moisturizer. Conclusions : The present study demonstrated that the high-resolution MR images and spectra of human skin from a high field NMR instrument could be applicable to evaluating the hydration state of the stratum corneum.

Keywords: ^{1}H magnetic resonance spectroscopy (MRS); Hydration

INTRODUCTION

It's well known that high-field MR systems have an increased signal-to-noise ratio (SNR), which is required for producing small field-of-view (FOV) images with improved

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spatial resolution. In accordance with this characteristic, a very high-field MR system such as a 14.1T is a likely modality for achieving high-resolution images *in vitro*. The higher SNR and improved tissue contrast that's obtained from a high-field system, when coupled with an optimized coil size, improves visualization of the morphological details in the human skin structure, and these details are not well seen at conventional field strengths. Particularly upon performing microscopy of the human skin, the experimental conditions are more difficult due to the short T2 relaxation times (~12ms) and the low effective proton density of the dermis, which is a structure that generates intense clinical interest.[1-5] However, Ablett and coworkers have performed high resolution MRI on the finger of a human subject with the use of high field MR systems and they showed that the water content in the skin could be measured.[6] Bittoun and coworkers were able to differentiate epidermis from dermis and they detected hair follicles *in vivo* using a whole body MRI system.[1] At present, MR microscopic imaging *in vivo* and *in vitro* has been applied to many organs with and without including skin. Using MR microscopy, Johnson and Maronpot performed toxicology studies in the liver,[10] and Morehouse and coworkers performed toxicology studies in the kidney.[11] Moller and coworkers employed MR microscopy to investigate the lungs in live animals with the use of hyperpolarized gases as contrast agents to enhance the signal in the airways.[12, 13] Furthermore, Hogers and coworkers obtained ultra high resolution images of fixed chicken embryos at several stages of development on a 17.6T.[14]

Although high field magnetic resonance technology has many merits, it also has intrinsic drawbacks to study skin hydration and skin permeation by drugs from dermal and transdermal formulations. To overcome these matters, it is possible to use a fixed gradient system reported by Blumich and coworkers.[15] The fixed gradient system is generally called GARField, standing for Gradient At Right-angles to Field, adopt permanent magnet with constant magnitude. A solenoidal radio-frequency (RF) coil is placed in the gap between two magnets so near horizontal magnetic field in the horizontal plane and a strong gradient of field strength in vertical direction, that is to say, the static polarizing magnetic field B0 and the rf field B1 are approximately orthogonal to each other. Based on the fixed strong gradient, it is possible to obtain high spatial resolution, of the order of 5-15 μ m, with very

short echo time, typically 50-200 μ s.[16] The cost is a degraded signal-to-noise ratio and, in practice, limitation to a single spatial dimension (profiling, not imaging).

The purpose of this work was to achieve high resolution MR imaging and spectra of human skin in vitro with using a 14.1 T MRI/MRS system, and to evaluate the hydration effect by comparing the spectra and verifying the usefulness of the MR system for diagnosing skin diseases and for developing cosmetic products.

MATERIALS AND METHOD

Subjects

A skin tissue sample for ^{1}H MRI/MRS was obtained from a cadaver by performing a routine autopsy. Fat layers were removed because that not only had an influence on measuring the effect of hydration but also caused an inconvenience of inserting a sample into small bore of NMR. A 7×7 mm size fresh skin tissue sample was obtained from the forearm and stored in a freezer by until required. Moisturizer was treated 50 μl per each sample using microroller. The sample was inserted in a glass tube (10mm diameter) for the RF coil. For measuring the hydration effect of a moisturizer, the ^{1}H MR spectroscopic data were obtained before and 3 hours after the application of moisturizer.

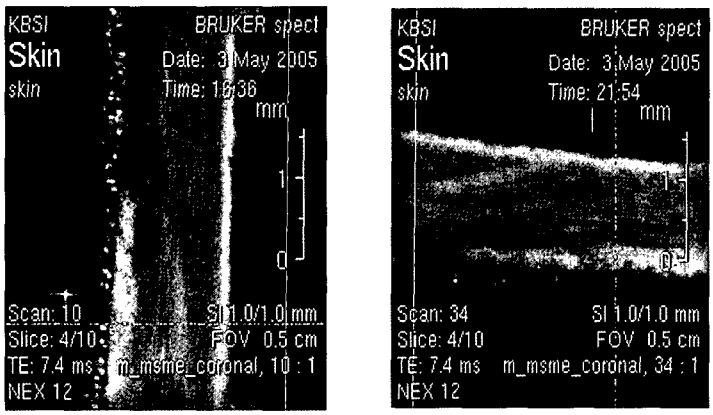
^{1}H Magnetic Resonance Spectroscopy

All the experiments were performed on a 14.1T vertical bore MRI/MRS system (Korea Basic Science Institute, Daejeon, Korea) with using a RF solenoid coil. The gradient strength was 4.8 G/cm/A. The inner diameter and outer diameter of the gradient coil were 19 and 40 mm, respectively. 1.3%, 1.6% and 2.1% linearities of the peak-to-peak ratios were 18 mm, 19 mm and 20 mm spheres, respectively. Spin echo and fast spin echo pulse sequences were employed for obtaining high resolution MR images of human skin. The image parameters were as follows: a 256 x 256 acquisition matrix, a 0.5 cm FOV, 12 NEX of acquisition, a TR/TE=600/7.7 ms and a 1.0mm slice thickness. The PRESS pulse sequence for ^{1}H MRS was used to obtain spatially localized volumes of 0.5/1.0 mm^{3} in human skin. In vitro ^{1}H MR spectra were obtained without water suppression to get a pure

water peak. The spectral parameters were the same before and after applying a moisturizer, i.e., a TR/TE=2000/10.46 ms and NEX=256. The voxel size was the only factor that changed when evaluating the SNR based on the voxel size. Since the spectra obtained before rubbing on a lotion had too low a SNR to estimate the quality of the spectra, it was multiplied 8 times by the original amplitude to analyze it more clearly.

Statistics

Statistical analysis was performed by using SPSS (Windows Version 6.0, SPSS Inc., Chicago, IL). The data were analyzed with independent sample *t*-tests for comparison of the two groups' data, the skin with applied moisturizer and the fresh skin. The paired-samples *t*-tests were used for the bilateral spectra within a group; P values <0.05 were considered significant to account for the multiple comparisons.



(A) (B)

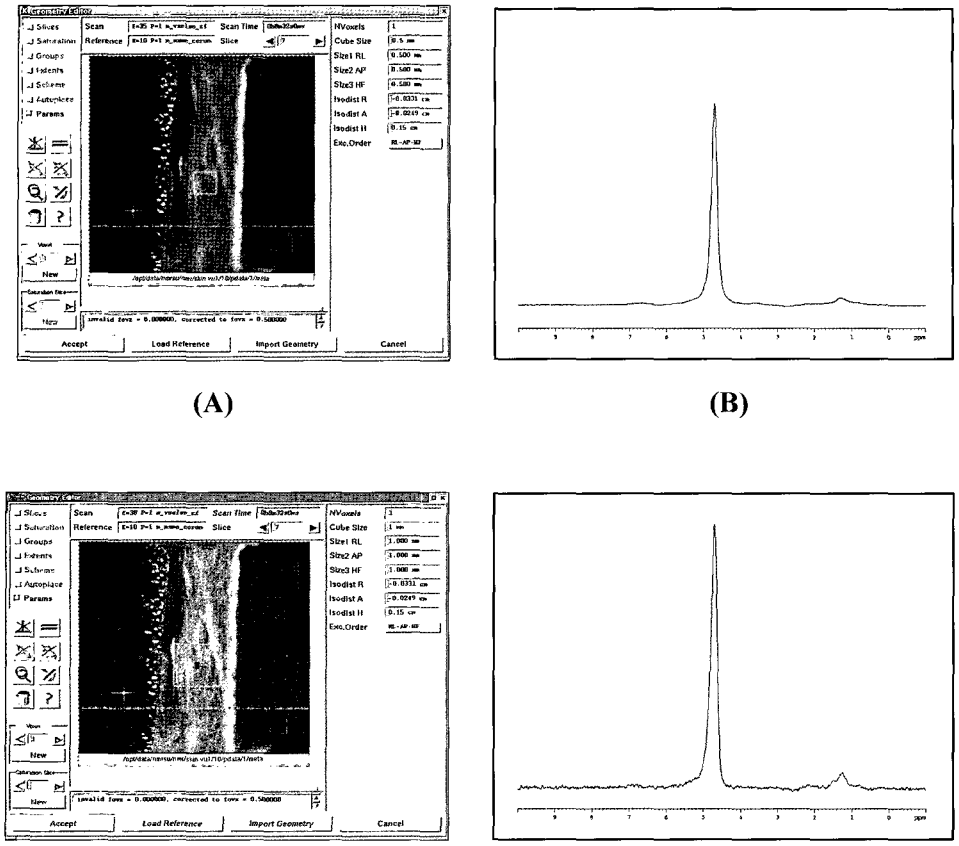
Fig. 1. High resolution MR images obtained on a 14.1 T system before applying a moisturizer (A) and after applying a moisturizer (B)

RESULTS

Fig. 1 shows the high resolution MR images that enabled a person to detect each component of human skin such as the epidermis, dermis, hypodermis and muscle. The outer bright thin layer indicates epidermis that's about 0.3 mm in thickness. The dermis is visible as a darker layer of about 1.0 mm thickness. The dermis is followed by the hypodermis, and it contains veins, arteries, eccrine sweat glands, etc. The SNR and the resolution of the spectra in accordance with the voxel size and location are represented in Fig. 2. The spectrum without water suppression displayed a significantly increased water peak (4.7 ppm) relative to a lipid peak (1.3 ppm). As we can see in Fig. 2A, the voxel size and location were 0.5 mm^3 and dermis, respectively. In order to analyze the effect of the voxel size, the images and spectra in Figs. 2C and 2D were obtained under identical conditions except for changing the voxel size from 0.5 mm^3 to 1.0 mm^3. As we expected, the signal intensity of the MR spectrum in Fig. 2D was significantly increased compared with that in Fig. 2B. Low signal intensity and poor resolution of the spectrum was obtained due to the signal contamination triggered by air and the small voxel size. After applying moisturizer, the signal intensity of the spectrum was substantially increased owing to the preserved moisture generated by the moisturizer.

DISCUSSION AND CONCLUSIONS

The high resolution MR images and spectra of human skin in vitro were successfully obtained on a 14.1T system. Although we treated the skin with a moisturizer resulting in increasing relaxation times, there were no significant image contrast variations between before and after rubbing the moisturizer. Thus, treating the skin with a moisturizer was not successful for distinguishing very precise structures, such as the cutaneous vasculature, the stratum corneum and hair follicles.



(A) **(B)**

(C) **(D)**

Fig. 2. Comparison of the MR images and MR spectra obtained before rubbing on a lotion with changing the voxel size and location. Each of the voxel sizes and locations are shown in Figs A and C. The corresponding MR spectra are shown in Figs B and D. The voxels were located on the dermis and near the epidermis, respectively. Each voxel had the same size, 0.5 mm^3 (A). The voxel was located on the dermis and the size was 1.0 mm^3 (C).

The water concentration of human skin after applying a moisturizer was higher than that before applying a moisturizer. Since the SNR is proportional to the voxel size, controlling the voxel size is critical for the quality of the MR spectra in vitro. It was thought that a moisturizer had a lipid component by the observation of an increased lipid peak, compared with the spectra obtained before applying a moisturizer. The epidermis has been considered to be the primary barrier for restricting transport across the skin.[17] Nowadays, commercial products such as scopolamine for motion sickness, nitroglycerin for heart disease and clonidine for premenstrual syndrome (PMS) are using transdermal delivery for passive diffusion of these compounds.[18] Putting it succinctly, the application of MRS techniques for measuring the biochemical change induced by scopolamine, clonidine and so on can be helpful for understanding the mechanism of drug delivery through the epidermis. The quantitative and spatially resolved knowledge of the hydration process of the outmost skin layer such as the epidermis, and especially the stratum corneum, is of great interest to the cosmetic industry.[19-20]

As compared with the results of the low field, static gradient relaxation analysis approach, i.e. GARField MR technology, high field spectroscopy having switched gradient also showed the effect of hydration process caused by the moisturizer. Although GARField was able to distinguish the epidermis from the stratum corneum based on have 6.5 μm of data point separation, high field MR image and spectroscopy could not detect the difference between stratum corenum and epidermis due to the limit of spatial resolution in the former and the limit of voxel size in the latter. One of the most important aspects is largely related to magnetic susceptibility increased according to the magnetic field strength. A plausible way to solve the matter on magnetic susceptibility is adopting a device, for example, a new sample mount platform with three level adjustment screws.[21] It will be helpful to stabilize field homogeneity and consequently enhance the spatial and spectral resolution. Also, to overcome the limitation of high field MR spectroscopy, there are some obstacles that needed to be resolved. Among many challenging factors, one is the voxel localization technique and the other is the signal processing skill. For placing the voxel on the epidermis, the voxel size should be adjusted to less than 0.5 mm^3. Furthermore, to place the voxel on stratum corenum, a much smaller voxel could be achievable. Yet at this time, as can be seen in Fig. 2, the voxel size of 0.5 mm^3 could be the minimal value to create enough signals for obtain-

ing high resolution MR spectra on a 14.1 T system. As a consequence, it is essential to develop localization and signal processing techniques simultaneously in order to obtain high resolution images and spectra that can be used in the clinical field. If possible, we recommend for researchers to make use of skin sample containing thick epidermis for easy localization of the voxel on the epidermis and for achieving less signal contamination by the outside air.

In conclusion, our present results show that high-field MR images and spectra have sufficient potential for expanding the field of clinical diagnosis in dermatology. The present study also demonstrated that high-resolution MR images and spectra of human skin from a high field NMR instrument could be used to evaluate the moisture concentration and the hydration effect. In addition, high resolution MR imaging has been used for the study of fMRI and the central nervous system with its improved spatial resolution and image contrast. It is ultimately possible that the MRI/MRS system will be used as a powerful tool that can be applied to the cosmetic and pharmaceutical industries.

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