Non-Invasive ESR Measurements for in vivo Kinetic Studies of a Nitroxide Radical in the Liver of a Rat by a Surface-Coil-Type Resonator under a Field Gradient

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A non-invasive ESR measuring method for in vivo kinetic studies of a nitroxide radical in the liver of a rat by combining a surface-coil-type resonator (SCR) and field gradient method was developed. The depth across which a microwave magnetic field (B_1) can penetrate under the SCR was about 4 mm. Using the SCR, ESR measurements without any invasive surgical procedures were made on the upper abdomen of a rat that received an intravenous injection of a nitroxide radical under a field gradient along the B_1-direction. It was found that the decay rate of nitroxide radical 4 mm under the skin (i.e., in the liver) is fast compared to that near the skin.

A surface-coil-type resonator (SCR) is one of the ESR resonators operating at the UHF band. It is composed of a single-turn coil and a transmission line, equivalent to an LC circuit, in which the former provides the inductance and the latter, the capacitance.[1,2] This resonator can be positioned in any of several possible sites in a living animal. Recently, we conducted in vivo kinetic studies of an intravenously injected nitroxide radical with a relative short half-life, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), at the liver, kidney, stomach, rectum, vein, and skin of rats, using SCRs.[3] From these measurements, one can estimate the reducing ability of each organ. For measurement of the liver and kidney, the target organs were exposed by laparotomy; then the SCR was placed on the surface of the organ. This experimental method required an invasive surgical procedure. It is more desirable to use a non-invasive method so that measurements can be made with the subject in a more natural physiological states. In the present study, we developed a non-invasive ESR measuring method for in vivo kinetic studies of a nitroxide radical in the liver of a rat by combining SCR with a field gradient method.

A scheme of the SCR is shown as Figure 1. The inner diameter of the single-turn coil of the SCR is 10 mm. This coil was constructed from copper wire measuring 0.3 mm in diameter. The length of the transmission line was calculated on the basis of the diameters of the coil and wire and the resonant frequency.[1,2] We had been using a flexible coaxial line as a transmission line because it is easy to handle.[3,4] However the insertion loss of semirigid coaxial lines is smaller than that of flexible lines. In this study, semirigid lines with a 50 Ω characteristic impedance was used to obtain a highly sensitive detection capability. A 700 MHz-microwave ESR spectrometer constructed at our laboratory (already described in detail[3–6]), consisted of a main electromagnet (with air-core, water-cooled, and of a two-coil Helmholtz design), a pair of field scan coils, a pair of field gradient coils, for x-, y-, and z-axes (maximum gradient, 1 mT/cm in a 20 mm range from the center), a pair of field modulation coils, power supplies, a personal computer, and a 700 MHz-microwave circuit for homodyne detection. The SCR was connected to a VSWR bridge of the microwave circuit. Conventional matching circuits were used to match the impedance of the SCR and input impedance (50 Ω) of the VSWR bridge at a resonant frequency of approximately 740 MHz.[2] The unloaded Q-value of the SCR under these conditions was 150.

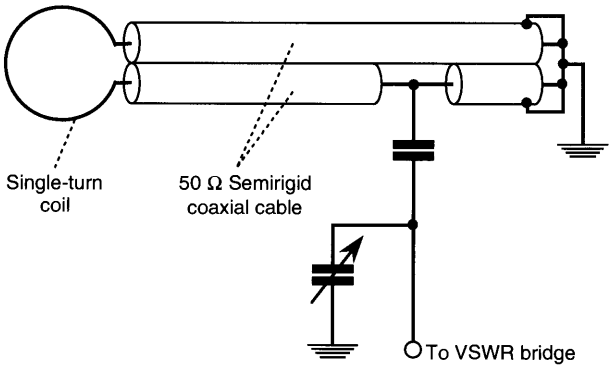

Figure 1. Schematic diagram of the SCR.

ESR measurements were performed when the SCR inserted into a TEMPOL aqueous solutions. The detection limit was estimated on the basis of the concentration of TEMPOL at a signal-to-noise ratio of 3dB. The detection limit of the SCR in this study was 50 μ mol dm^{-3}, smaller than that of our previous SCR, in which the transmission lines were constructed of flexible coaxial lines.[3] Figure 2 shows the two-dimensional ESR images projected on the zx-planes of the SCR that was placed on the surface of a cylindrical phantom (diameter, 30 mm; axial

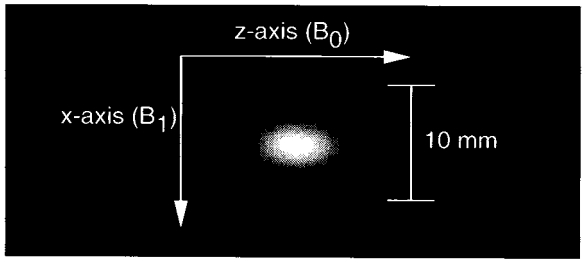

Figure 2. Two-dimensional ESR images of TEMPOL projected on the zx-planes of the SCR that was placed on the surface of a cylindrical phantom (diameter, 30 mm; axial length, 10 mm). The visualized area indicates the radiation space of B_1.

length, 10 mm). The phantom consisted of agar that included a physiological saline solution (a 0.9% sodium chloride aqueous solution) and TEMPOL (final concentration, 10 mmol dm^{-3}). The z- and x-axes represent the directions of the static (B_0) and alternating (B_1) magnetic fields, respectively. The visualized area is the radiation space of B_1. The full-width at half-maximum of the signal intensity (i.e., B_1 strength) along the x-axis was about 4 mm.

Under sodium pentobarbital anesthesia, a male Wistar rat weighing 260 g was restrained in the B_0 field. The single-turn coil of the SCR was placed on the shaved skin of the upper abdomen without using an invasive surgical procedure. Because the thickness of the skin and muscle above the liver is about 2.5 mm, a sufficiently strong B_1 field can reach the liver. In this condition, the loaded Q-value of the SCR was 120. The rat received 1 cm^3 of a 0.4 mol dm^{-3} TEMPOL solution, which has been dissolved in physiological saline solution, via the tail vein and the ESR measurements were started. The measurements were repeated every 7 s from 6 s to 118 s with a field sweep width of 1.5 mT, observing only at the lowest component of the hyperfine triplet, under a field gradient of 0.5 mT/cm along the x-axis. One spectrum was obtained from 6 averages at a scan speed of 1.25 mT/s.

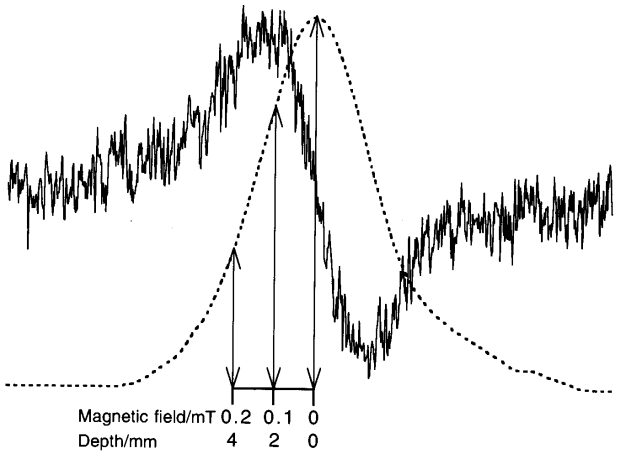

Figure 3. An example of ESR spectra taken from the upper abdomen of a rat that received intravenous injection of TEMPOL (solid line, raw data; dashed line, integrated data). The signal intensities at a point located 0, 2, or 4 mm under the SCR (i.e, in a magnetic field of 0, 0.1, or 0.2 mT lower than at the center) were measured from the integrated spectra.

An example of ESR spectra obtained is shown in Figure 3. The signal intensities of the point at a distance of 0, 2, or 4 mm under the SCR (i.e, in a magnetic field of 0, 0.1, or 0.2 mT lower than at the center) were measured from the integrated spectra. Figure 4 shows plots of signal intensity at each point against time after the injection of TEMPOL. Because linearity was observed on the semilogarithmic plots, the half-life was used as a parameter to estimate the decay rate of TEMPOL. The half-life at 0, 2, and, 4 mm under the SCR was 88.8, 78.6, and 47.9 s, respectively, indicating that the half-life is shortened as the depth from the surface of skin increases. We already

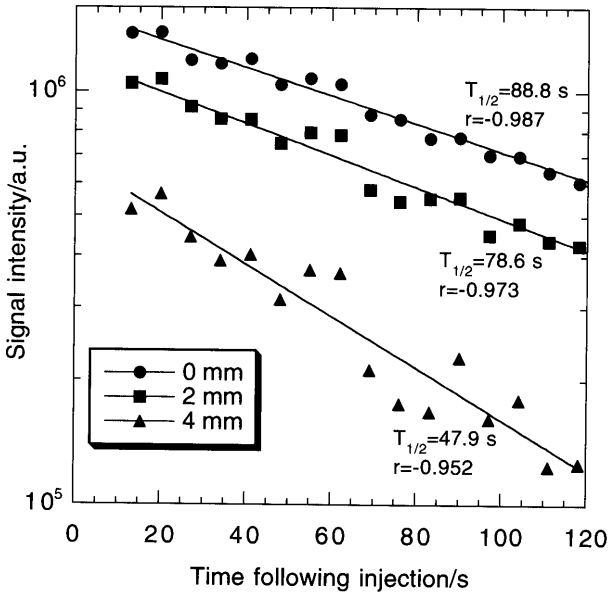

Figure 4. Semilogarithmic plots of the ESR signal intensity of TEMPOL, which was detected on the upper abdomen of a rat, against time after the injection of TEMPOL. The 0, 2, or 4 mm indicates the depth below the surface of skin.

measured the half-lives of TEMPOL on the liver (which was exposed by the laparotomy) and skin, and found that the half-life on the liver was shorter than that on the skin.[3] This finding suggests that the decay rate of TEMPOL in the liver is faster than that in the skin and/or muscle. This is consistent with the results in the present study, i.e., the half-life of TEMPOL 4 mm under the skin (viz., in the liver) is shorter when compared with that near the skin.

In this study, we conducted temporal ESR measurements at the upper abdomen in a living rat following the administration of TEMPOL by employing SCR under field gradient. The method demonstrated here can simultaneously provide information on the half-life of TEMPOL in the liver and skin (and/or muscle) without employing any invasive surgical procedures.

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