

Reduction of Carcinogenic Chromium(VI) on the Skin of Living Rats

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The reduction of chromate (Cr(VI)) on the skin of living rats was investigated using the technique of *in vivo* electron paramagnetic resonance (EPR) spectroscopy with a surface coil resonator. Topical application of aqueous solution of Cr(VI) on the skin of rats generated the transient species, Cr(V). Partial removal of the stratum corneum increased the rates of formation and decay of Cr(V), as well as Cr(V) signal intensity. The results demonstrate that skin represents one route for chromium to enter into animals and humans. The generation of reactive Cr(V) species in the reduction of Cr(VI) by the skin may play an important role in the mechanism of Cr(VI)-induced skin cancer. These findings indicate the potential for *in vivo* EPR spectroscopy for studying the metabolism of paramagnetic reactive species in chemical and biochemical reactions occurring in/on the skin of both small and large animals, and possibly humans.

Key words: chromium; *in vivo* EPR; skin.

INTRODUCTION

Chromate (Cr(VI)) compounds, widely used in industry, have been shown to have serious toxic and carcinogenic effects in humans. Cr(VI) has been demonstrated to induce a variety of DNA lesions such as single-strand breaks, alkali-labile sites, and DNA protein cross-links. Since Cr(VI) does not react with isolated DNA, the reduction of Cr(VI) by cellular reductants to lower oxidation states has been considered to be an important step in the mechanism of Cr(VI)-induced carcinogenesis (1). Because Cr(V) complexes are generally characterized as being labile and reactive, whereas Cr(III) complexes are relatively nontoxic, noncarcinogenic, and nonmutagenic (1–3), the detection of Cr(V) formation from microsomal reduction of Cr(VI) led to the suggestion that the Cr(V) intermediates are likely candidates for the "ultimate" carcinogenic form of chromium compounds (4).

Cr(V) species have been reported to be generated in the reduction of Cr(VI) by various biological systems, includ-

ing microsomes (4, 5), mitochondria (6), human red blood cells (7), and rat thymocytes (8). Detection of the electron paramagnetic resonance (EPR) signal of Cr(V) has also been reported in isolated liver cells and red blood cells of 14-day chick embryos after treatment with Cr(VI) (9). Very recently, using a newly developed low frequency EPR spectrometer equipped with a cylinder-shaped loop gap resonator, we were able to show that Cr(V) intermediates were generated in the liver and kidney of whole living mice after intravenous administration of Cr(VI) (10, 11).

In addition to inhalation as the main route for occupational exposure, skin represents another route for chromium to enter into humans and to exert its carcinogenicity (12). Cr(VI) is known to be readily absorbed through human skin (13). Chronic ulcers of the skin and acute irritative dermatitis have been reported consistently in workers exposed to chromium-containing materials (14). It has been reported that, when rats were administered Cr(VI) compounds subcutaneously, fibrosarcomas developed at the injection site (15, 16). It is the purpose of present work to determine whether Cr(VI) is reduced to Cr(V) after topical application to the skin, and whether the skin barrier has an effect on the rate of the reduction process. To detect the formation of Cr(V) in the skin of living rats, we used a low frequency EPR spectrometer equipped with a surface coil resonator, which was recently developed in our laboratory.

MATERIAL AND METHODS

Materials

Sodium dichromate (Cr(VI)) and α,α' -diphenyl- β -picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO) and used without further purification.

Animal Preparation

Wistar rats weighing 200–220 g were obtained from Charles River Laboratories (Wilmington, MA). After the animals were anesthetized with ketamine/xylazine (100/10 mg/kg, intramuscularly), the hair on the back of the rat was shaved to expose the skin. Then 200 μ l of 0.1 M aqueous solution of sodium dichromate were applied topically to the skin. A thin plastic foil was used to cover the shaved area to maintain humidity and also to prevent contamination by dichromate of the detection coil of the EPR resonator. To study the effect of skin barrier on the reduction of Cr(VI), in a group of animals the stratum corneum of the skin was removed by stripping with surgical tape 10 times before application of dichromate.

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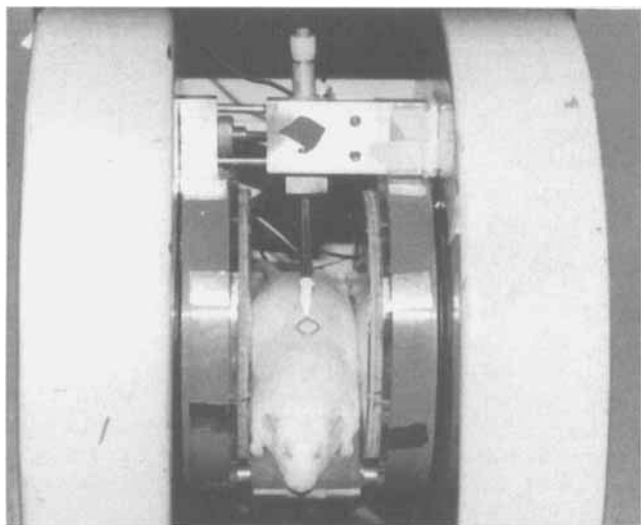


FIG. 1. A picture of a rat in a 4-inch wide gap magnet with an extended surface coil resonator positioned over the dichromate treated skin. The EPR spectrometer operated at a microwave frequency of 1.2 GHz.

This procedure of stripping is a common practice for removing the stratum corneum. No histological study was carried out for the stripped skin in this study, but according to our experience from previous experiments, the correct degree of stripping was estimated from the change of the appearance of the skin (it becomes shiny).

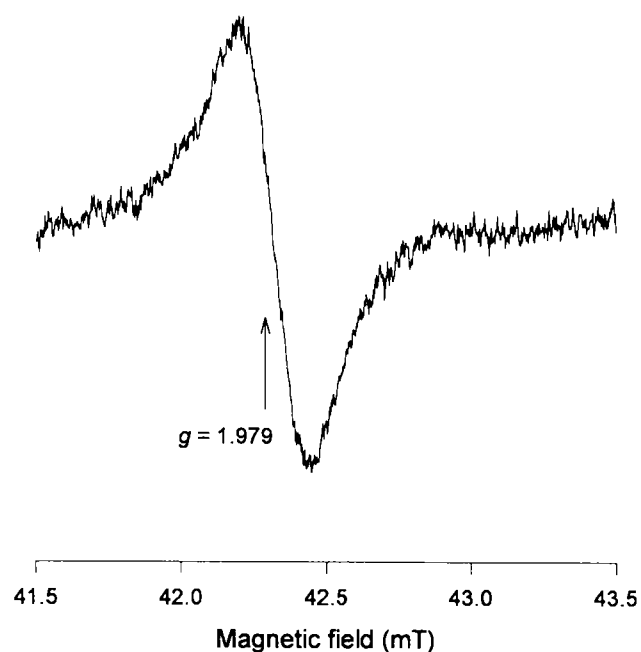


FIG. 2. L-band (1.2 GHz) EPR spectrum of Cr(V) obtained from the skin of a living rat. The spectrum was recorded 30 min after 200 μ l of 0.1 M aqueous solution of sodium dichromate were applied topically to the skin. Spectrometer settings included magnetic field, 42 mT; incident microwave power, 50 mW; scan time, 300 s; modulation amplitude, 0.16 mT. The g factor of Cr(V) was measured using DPPH as a standard.

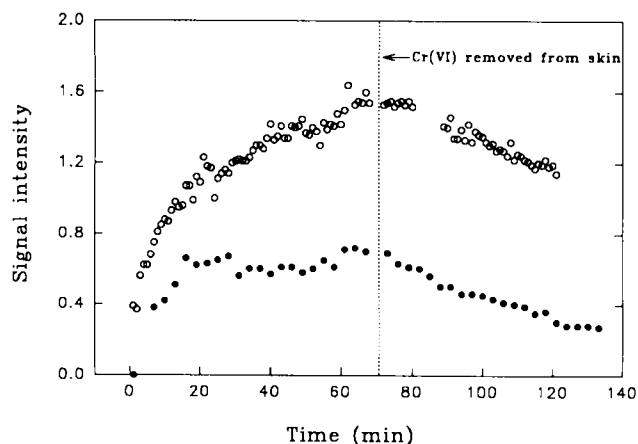


FIG. 3. A typical time course of the changes of Cr(V) signal intensity. The EPR signal was scanned repeatedly immediately after the application of the sodium dichromate solution to the skin. Time zero is the time of application. (○) Skin was shaved, and stripped 10 times with surgical tapes; (●) shaved skin without stripping. Cr(VI) was removed from the skin by washing with tap water.

EPR Measurements

In vivo EPR measurements of Cr(V) on the skin of living rats were obtained using a custom-designed EPR spectrometer with a low frequency (1.2 GHz, L-band) microwave bridge and an extended loop resonator (Fig. 1). After dichromate was applied to the skin of the rat, the animal was immediately placed between the poles of the magnet with the coil of the extended loop resonator positioned on top of the skin of interest. The EPR spectra were collected every 60 s to record the formation and decay of the Cr(V) signal. Typical settings for the spec-

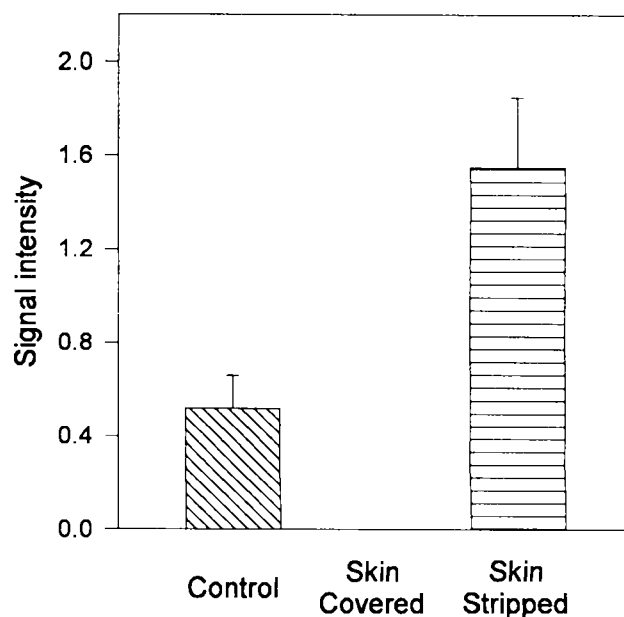


FIG. 4. The effects of covering the skin and removal of stratum corneum on the signal intensity of Cr(V). The signal intensity was measured from the peak-to-peak height of the Cr(V) signal obtained 60 min after application of Cr(VI) to the skin. The values represent means \pm SE ($n = 3$).

trometer included magnetic field, 42 mT; incident microwave power, 50 mW; scan time, 60 s; modulation amplitude, 0.16 mT. The EPR spectra were collected, stored, and manipulated using a home designed software installed on IBM-compatible computers. The g factor of Cr(V) was calculated using DPPH crystals as a standard.

RESULTS AND DISCUSSION

After application of an aqueous dichromate solution to the skin of a living rat, an EPR signal was observed from the treated area (Fig. 2). The EPR signal of the spectrum was a single line with a line width of 0.25 mT, and a g factor of 1.979. Since Cr(VI) is EPR silent, and the parameters of the recorded EPR signal are identical to those reported in the literature for Cr(V), we assign this signal to Cr(V). This result provides the first direct evidence on the reduction of Cr(VI) to Cr(V) in the skin of the living rat. The result also demonstrates that skin represents one of the routes for chromium to enter into animals. The reduction to Cr(V) began shortly after application of Cr(VI) to the skin and continued for at least 60 min.

To verify that the generation of Cr(V) was the result of reduction of Cr(VI) occurring in the skin of the animals, the following control experiments were performed. (a) No EPR signal was observable in the Cr(VI) solution, nor in the skin before treatment; (b) if the dichromate solution was applied to a thin plastic film, which was placed over the shaved skin to prevent direct contact of the dichromate with the skin, no EPR signal of Cr(V) was observable; and (c) intensity of the Cr(V) signal decreased and then dropped to zero when the treated area was gradually moved away from the detection coil. These results indicate that Cr(V) was formed through reduction of Cr(VI) in the skin, not due to a chemical reaction occurring in the aqueous solution, or to other artifacts.

Figure 3 shows a typical example of the kinetics of the formation and decay of Cr(V) signal. As can be seen, the signal intensity grew with time and then reached a plateau. Upon removing of the Cr(VI) from the skin by washing with tap water, the signal decayed with time. Also shown in Fig. 3 is the effect of the skin barrier on the kinetics. Apparently, removing the stratum corneum (by stripping) increased the rates of both formation and decay of Cr(V). In addition, removing of the skin barrier also significantly increased the intensity of Cr(V) signal when compared with the control, as shown in Figs. 3 and 4.

The importance of the stratum corneum as a diffusion barrier was evident because a higher rate of formation of Cr(V) species and the significantly increased signal intensity of Cr(V) were observed in experiments with removed stratum corneum (by stripping). These results are in agreement with the expectation that the reduction of Cr(VI) in skin requires that Cr(VI) reach functional cells.

Cr(V) has been shown to be capable of generating OH[•] radicals via Fenton-like reaction (17), and these reactive species can cause DNA damage. Since Cr(VI) can be readily absorbed through the skin (13), and then reduced to Cr(V) as demonstrated in this study, Cr(V) and its

related OH[•] radical generation are likely to play an important role in the mechanism of Cr(VI)-induced carcinogenicity. These results are consistent with reports that when rats were subcutaneously administered a Cr(VI) compound, fibrosarcomas developed at the injection site of the animals (15, 16).

The results in the present study also illustrate that *in vivo* EPR spectroscopy can be a very useful and powerful tool for studying paramagnetic reactive species in chemical and biochemical reactions occurring in/on the skin of both small and large animals. Because this area is near the surface of animals, it permits the technique of *in vivo* EPR to exploit its unique advantage of directly studying the paramagnetic species itself, and at the same time, avoiding the sensitivity problems usually associated with the limited depth of penetration of microwaves at L-band frequency (~1–2 GHz). Consequently, the size of the subject being studied becomes irrelevant, and the technique can be readily applied to both small and large animals, and potentially, to humans.

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