Inhibition of Human Peripheral Blood Neutrophil Respiratory Burst by Alcohol-Based Venipuncture Site Disinfection

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Ethanol inhibits the respiratory burst of neutrophils. Therefore, the effects of alcohol-based skin disinfection on oxygen metabolism in neutrophils were tested using 70% ethanol or an ethanol–isopropanol–n-propanol mixture. Neutrophil respiratory burst activity as assessed fluorometrically by oxidation of 2',7'-dichlorofluorescein diacetate increased at 10 min after disinfection with 70% ethanol compared to the activity at 30 s. The increase was significant for triggering oxidative burst with formylpeptide but not with phorbol myristate acetate.

Clinically, the most important neutrophil defects involve chemotaxis, respiratory burst, and intracellular microbial killing. Defective generation of the respiratory burst with consequent impaired microbial killing defines chronic granulomatous disease and its variants as well as glutathione peroxidase deficiency (16). In clinical testing of respiratory burst, nitroblue tetrazolium has been adapted to automated measurements; furthermore, colorimetric tests, luminol-isoluminol-amplified chemiluminescence, and fluorometric assays have been developed (3). Quantitative alterations of neutrophil respiratory burst are reported for investigational and clinical topics (see for example, references 1 and 12). In alcoholic patients, a defect was discovered in the oxidative metabolism of neutrophils in the presence of ethanol (6, 8–10). Ethanol inhibited the influx of calcium from the extracellular fluid, which could explain an observed reduction of the generation of diacylglycerols and inhibition of the oxidative reaction in neutrophils (9).

Skin sterilization for a venipuncture is routinely done with commercially available disinfectants for preventing both nosocomial infections of patients and bacterial contamination of blood culture samples (2, 4, 5, 13–15). Use of iodine tincture appears to be at least as effective as alcohol in preventing bacterial contamination of blood samples (2) and may be superior to povidone-iodine for venipuncture site antiseptic (5, 14). Alcohol-based antiseptics improve the bactericidal activity of iodine and decrease the amount of time needed for skin disinfection (4).

We hypothesized that measurement of respiratory burst activity of neutrophils from peripheral blood may be affected by contamination of blood samples with alcohol used for disinfection of venipuncture sites. Therefore, the effect of alcohol-based skin disinfection on stimulated oxygen radical release from neutrophils of healthy donors was tested in a systematic manner.

Two disinfectants, namely, 70% ethanol (EtOH) and a commercially available mixture of 10 to 25% EtOH, 25 to 50% isopropanol, and 25 to 50% n-propanol (IPOH) (Arcana, Spittal/Drau, Austria), were compared for their effects on neutrophils after potential sample contamination. Eight healthy volunteer physicians participated in the study after giving informed consent. Two designated areas each of the right and the left forearm were sterilized with EtOH or IPOH in a predefined uniform fashion by two scrub procedures using alcohol-satured pads. Then, 4 ml of blood was drawn simultaneously at each of two sampling times, 30 s and 10 min after the sterilization procedure. The two time periods were chosen to allow different evaporation times. Neutrophils were isolated from the blood samples (anticoagulated with EDTA at 1.6 mg/ml of blood) by dextran sedimentation and centrifugation through a layer of Ficoll-Hypaque, followed by hypotonic lysis of contaminating erythrocytes using sodium chloride solutions as described elsewhere (12). Neutrophil respiratory burst activity was measured by an assay using 2',7'-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, Oreg.). This assay is based on the oxidation of nonfluorescent DCFH-DA to highly fluorescent 2',7'-dichlorofluorescein (DCF) both intracellularly and extracellularly. Neutrophils (8 × 10⁶ cells/ml) were resuspended in phenol-red-free Hanks balanced salt solution (HBSS) without Ca²⁺ and Mg²⁺ (Gibco-BRL Life Technologies, Vienna, Austria). Then, 2 × 10⁵ polymorphonuclear leukocytes at 100 µl/well (96-well plate; Falcon 3072) were immersed in a 10⁻⁶ mol/liter solution of DCFH-DA in phenol-red-free HBSS without Ca²⁺ and Mg²⁺ containing 1 µmol/liter of formyl-methionyl-leucyl-phenylalanine (fMLP) or 10 µmol of phorbol myristate acetate (PMA) (both from Sigma Chemical Corp., St. Louis, Mo.) per liter as a triggering agent.

The plates were placed in a humidified incubator at 37°C (5% CO₂) for 10 min as described previously (12). Fluorescence activity given as arbitrary units (AU) was determined at 485/20-nm excitation and 530/25-nm emission wavelengths using the CytoFluor Multi-Well Plate Reader Series 4000 (PerSeptive Biosystems, Inc., Framingham, Mass.). Data are expressed as the mean and the standard error of the mean (SEM). Means were compared by two-tailed paired Student t test and Friedman analysis of variance. Statistical analyses were performed using the StatView software package (Abacus Concepts, Berkeley, Calif.).

Peripheral blood neutrophils obtained after skin sterilization with two alcohol-based disinfectants and two different evaporation periods before venipuncture showed an impaired generation of oxygen radicals, as measured fluorometrically (Fig. 1). The highest amounts of fMLP- or PMA-triggered oxygen radicals of neutrophils were observed in blood samples that
were drawn 10 min after skin disinfection with EtOH. Neutrophils from samples drawn 30 s after EtOH disinfection exhibited a statistically significant 30% reduction in fMLP-triggered oxygen radical production. The PMA-triggered response was diminished by 22%, which was not statistically significant. In contrast, disinfection of venipuncture sites with IPOH reduced oxygen radical production in a more-pronounced fashion and independent of the sampling time or the stimulus; after IPOH disinfection, the levels of oxygen radical production did not rise when the evaporation period increased from 30 s to 10 min either after fMLP triggering or after PMA triggering. At 10 min after IPOH disinfection, the respiratory burst activity levels remained significantly lower than after EtOH disinfection (Fig. 1).

In this report, we have shown that alcohol-based skin disinfection of venipuncture sites for peripheral blood sampling may affect the respiratory burst activity of neutrophils. By obtaining blood samples by venipuncture immediately (30 s) after EtOH disinfection, the inhibitory effect was evident, since after a longer period of time (10 min) between disinfection and venipuncture, oxygen radical release was more pronounced, possibly due to better alcohol evaporation. The inhibition of respiratory burst appeared to be stronger when the propanol-containing mixture was used as a disinfectant.

Previous studies have shown that ethanol inhibits the production and secretion of oxygen products from neutrophils in vitro (6, 8–10). This effect was observed for respiratory burst activity triggered with both fMLP (8), which acts via cell surface receptors, and PMA (6), which stimulates respiratory burst intracellularly by activation of protein kinase C (17). The ethanol concentrations tested and found to be effective in those in vitro experiments were between 0.1 and 1.0%. However, due to contamination after skin disinfection, such ethanol concentrations are unlikely to be reached in a 4-ml blood sample. Nevertheless, more recent reports have shown that inhibitory effects on in vitro functions of neutrophils may well be observed at an ethanol concentration that is up to 100-fold lower, i.e., at clinically relevant amounts (11). Theoretically, such concentrations may have been achieved in our study by contamination, especially with the shorter evaporation period. In addition, the observation of inhibition of neutrophil respiratory burst after propanol disinfection suggests that propanol also affects neutrophil function, which has not been described so far. Overall, the inhibitory effect on neutrophil respiratory burst appeared to be less pronounced for EtOH than for IPOH, since at the 10-min sampling period, after disinfection with EtOH but not with IPOH, the respiratory burst activity of neutrophils was at its highest and, therefore, served as a reference value for statistical comparison. No time dependency was noted for IPOH. Whether other skin disinfectants, such as iodine or chlorhexidine, exert similar effects on neutrophils when used at the venipuncture site has not yet been reported.

Several examples exist that illustrate contradictory findings for quantitative neutrophil functions in clinical pathology (7), possibly indicating variabilities in testing. The results of the present study suggest that this variability not only may be due to disease pathology or to treatment effects but also may be related to influences of skin disinfection procedures which, until now, have not yet been considered to contribute to inter-assay and interlaboratory variabilities in neutrophil respiratory burst measurements. In summary, alcohol-based venipuncture site skin disinfection may differentially impair neutrophil respiratory burst. Direct comparison of ethanol disinfectant with an isopropanol mixture suggests that the best results are obtained with ethanol after sufficient time is allowed for evaporation before venipuncture.

**REFERENCES**

