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BLOOD TUBING AND CYTOKINE PRODUCTION: EFFECT OF STERILIZATION

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ABSTRACT

Blood tubings commonly represent an integral component of hemodialysis circuits. Different factors may influence their biocompatibility, such as the type of material, the sterilization mode and the geometry. In vivo the final biocompatibility may be further complicated by the individual host response, the flow parameters, and the impact of mechanical trauma on blood’s cellular components (i.e. erythrocytes). In this in vitro study we evaluated some commercially available blood tubings sterilized by different methods as to their interaction with normal leukocyte population and tested the response of these cells in terms of cytokines (IL-1β, IL-1Ra, TNF-α). As a positive control, leukocytes were incubated with 0.5 ng/mL of bacterial lipopolysaccharide (LPS) or with Cuprophan of comparable surface. The results showed that cytokine production was markedly reduced, particularly in the case of γ-ray-sterilized tubings. Of interest, it was not always related to the adherence. However in some cases, particularly of γ-ray sterilization, adherence was none, despite the cytokine production.

Key Words: Apoptosis; Biocompatibility; Cytokine; Tubing
INTRODUCTION

Continuing research in the synthetic polymers is amply justified for the large variety of biomedical applications for which they are already in use, such as the extracorporeal substitutive treatment for chronic renal failure. The physicochemical and mechanical characteristics of such polymers are generally designed to be appropriate for their proposed function, but the polymers do not necessarily possess optimal biocompatibility. An additional factor that is important as much as often overlooked is the influence of sterilization. More specifically in regard to the scopes of the present investigation, a growing trend toward substitution of ethylene oxide (ETO) as sterilizant with the adoption of y-ray radiation has been witnessed during the past years. This has occurred in the field of hemodialysis disposables and of tubings in particular. The “hemocompatibility” of the extracorporeal system is a complex issue that depends on a large variety of factors that may act in concert or individually. These include the dialysis membrane, the dialysate and the blood tubing. In the case of a low level of hemocompatibility, the chronic type of the hemodialysis therapy provides the basis for a repetitive stimulation of cytokine production leading to chronic inflammation.

Several studies have implicated the role of cell adhesion in the triggering of cytokine production (1,2). Cell adhesion has been shown to occur on dialyzer membranes and blood tubing depending on the polymer with the involvement of confounding factors such as the occurrence of endotoxin trace contamination (3), physiological stress (4) and the type of sterilization (5).

Blood tubing has historically drawn certain interest as to their possible adverse effect due to the leaching of plasticizers (6–8). The information regarding bioincompatibility are however scanty (5,9). Sterilization could theoretically induce clinically relevant adverse reaction either directly (i.e. residues) or indirectly (chemical-physical polymer alterations with potential release of degradation products).

In the present study, we evaluated the effect of different, commercially available blood tubing on cytokine production and leukocyte adhesion.

METHODS

Materials

Ethylene oxide- or y-ray-sterilized blood lines of polyvinyl chloride (PVC) were obtained from different manufactures (Cobe, Denver, USA, Bellco, Mirandola, Italy; Fresenius AG, Bad Homburg, Germany; Hospal, Medolla, Italy; Miramed, Mirandola, Italy). RPMI 1640 pH 7.4, LPS of E. Coli and p-nitro phenylphosphate (p-NPP) were purchased from Sigma (St. Louis, Missouri, USA). For the leukocytes purification the polypropilene tubes with septum and separation medium (LSM1077) were obtained from
FAR s.r.l. (Verona, Italy). Commercial ELISA kits for TNF-1-α, IL-1β, IL-1Ra (R&D System, Minneapolis MN, U.S.A.) were used. Triton X was obtained from BDH Chemicals (Lot, Poole, England). PBS buffer pH 7.2 was purchased from Pierce (Rockford, Illinois, USA).

**Analytical Procedures**

Bloodlines were cut in pieces with the same surface and divided in two groups. Ten tubing pieces were filled with human blood of volunteers and diluted (1/1, V/V) with a tissue medium culture RPMI (Figure 1) and another ten with purified leukocytes (Figure 2). Leukocytes were purified with sequential centrifugation followed by osmotic shock to eliminate contaminating erythrocytes (1). Blood was drawn into the tube and the leukocytes separation medium added. (35/15, V/V). The mixture was centrifuged at 1100×g for 20 minutes and then at 1300×g for 10 minutes at 4°C. The cells were washed 2 times with Tyrode’s buffer Ca²⁺ free, centrifuged at 1400×g for 10 minutes and then resuspended in RPMI. Leukocytes were counted using a standard hemocytometer. Negative control was blood added to RPMI and positive controls were blood in presence of LPS of E. Coli (0.5 ng/mL final concentration) and blood with Cuprophan sheet membrane with the same area. All samples and controls were incubated in static condition for 3 hours at 37°C. At the end we measured the plasma levels of TNF-α and IL-1β and IL-1Ra production (after cell lysis with repeated freeze-thaw cycles). The detection limits of TNF-α, IL-1β, IL-1Ra were 4.4 pg/mL, 1 pg/mL, 22 pg/mL respectively. The cytokines levels were referred to a normalized leukocytes population of 106 cells. Finally the tub-

![Figure 1. Schematic representation of the experimental procedure.](image-url)
ings were treated with Triton X, in order to remove all leukocytes that remained adherent on the internal surface of the tube. Tubing samples were rinsed with PBS buffer. Leukocytes adherence was measured by the acid phosphatase activity, using p-NPP as non-specific substrate that is hydrolyzed to p-nitrophenoxide (p-NP) and inorganic phosphate (3). The entity of the color development was referred to the cell number obtained from a standard curve. The data were referred to three experiments performed in duplicate.

**Statistical Methods**

Values were expressed as means ± standard deviation of the mean. Paired or unpaired Student’s t test was applied with \( p < 0.05 \) as significant.

**RESULTS**

The cytokine production induced by the static direct contact of normal human whole blood greatly varied with the different membranes tested without a significant difference between the different sterilization modes (ETO vs gamma, \( p > 0.05 \)) for TNF-\( \alpha \). (ETO: 202.6 ± 151; \( \gamma \)-ray: 205 ± 58, ETO vs. \( \gamma \)-ray, \( p > 0.05 \)), IL-1\( \beta \) (ETO: 40.8 ± 5.9; \( \gamma \)-ray: 38.2 ± 8.1, ETO vs. \( \gamma \)-ray, \( p > 0.05 \)), IL-1Ra (ETO: 1932.2 ± 654.3; \( \gamma \)-ray: 2046.3 ± 890.4,

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**Figure 2.** TNF-\( \alpha \) was evaluated in the plasmas of whole blood after static incubation in blood tubings (see Figure 1). IL-1\( \beta \) and IL-1Ra were determined in the cell lysates of purified leukocytes (see Materials and Methods). Leukocyte adhesion was investigated using a colorimetric assay (4).
ETO vs. γ-ray, $p > 0.05$) (Figure 3). Of note, cuprophan membranes induced the production of cytokines comparable with that observed with both ETO- and γ-ray-sterilized blood tubings. The large variation was not due to the individual response since all the blood used in the experiments was drawn from the same donor. Leukocyte adherence, as detected with a colorimetric assay, also showed no difference between ETO- and γ-ray-sterilized blood tubings (ETO: 8670.2 ± 6283; γ-ray: 2365 ± 2702, ETO vs. γ-ray, $p > 0.05$) (Figure 4). However, a significant inverse correlation was found between intracellular IL-IRa and adhesion ($R^2 = .049$) (Figure 5). Of interest, in...
two types of commercially available blood tubings we found no leukocyte adherence despite the fact that all the studied cytokines were produced.

**DISCUSSION**

The results of the present study show that blood tubing can induce cytokine production and release independently of the sterilization mode. There was no significant difference between ETO- and γ-ray-sterilized blood tubings. The levels of cytokine production were the same as those detectable when whole blood was incubated with cuprophan membranes. The cytokine produc-

**Figure 4.** Leukocyte adhesion on blood tubings as detected by a colorimetric assay (4).

**Figure 5.** Regression analysis between the number of adherent cells and the production of IL-1Ra. Each point represents the mean of three experiments performed in duplicate.
tion was related to the ability of the surface to induce adhesion, a prerequisite for the triggering of protein synthesis by leukocytes. It is therefore of concern that none of the commercially available blood tubings from different manufacturers are devoid of cytokine-inducing properties. This effect was not modified nor improved by X-ray sterilization, as often considered a “more biocompatible” approach for medical devices than ETO sterilization. The present studies were performed in experimental conditions in which flow was omitted. It is therefore likely that the leukocyte adhesion may have been overestimated in our in vitro assay in respect to the “normal” situation of use. However, although we agree that in our system leukocyte adhesion may have been overestimated, it is worth underemphasizing that the process is the prerequisite for the triggering of protein synthesis in leukocytes. Furthermore, it is also noteworthy that leukocyte adhesion was inversely correlated with the production of intracellular IL-1Ra. Therefore, cytokine production may be related to the extent of leukocyte adhesion, a prerequisite for leukocyte activation. In this context, two of the commercially available blood tubings showed cytokine production comparable to the other tubings but the leukocyte adhesion was almost negligible. At present, we have no explanation for this observation. However, we hypothesize that some tubings may induce cell-programmed death (so called apoptosis), a marker of leukocyte activation. Further experiments are needed to confirm this possibility.

REFERENCES


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