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Protective activity of propofol, Diprivan® and intralipid against active oxygen species

M. Mathy-Hartert,[1,CA] G. Deby-Dupont,[1,2] P. Hans,[2] C. Deby[1] and M. Lamy[1,2]

[1]Centre for the Biochemistry of Oxygen, Institut de Chimie, B6a, Domaine Universitaire du Sart Tilman, 4000 Liège; [2]Department of Anaesthesiology and Intensive Care, Centre Hospitalier Universitaire, B35, Domaine Universitaire du Sart Tilman, 4000 Liège, Belgium

[CA]Corresponding Author
Tel: (+32) 4 3663360
Fax: (+32) 4 3662866
Email: mmathy@ulg.ac.be

We separately studied the antioxidant properties of propofol (PPF), Diprivan® (the commercial form of PPF) and intralipid (IL) (the vehicle solution of PPF in Diprivan®) on active oxygen species produced by phorbol myristate acetate (10^{-6} M)-stimulated human polymorphonuclear leukocytes (PMN: 5×10^5 cells/assay), human endothelial cells (5×10^5 cells/assay) or cell-free systems (NaOCl or H_2O_2/peroxidase systems), using luminol (10^{-4} M)-enhanced chemiluminescence (CL). We also studied the protective effects of Diprivan® on endothelial cells submitted to an oxidant stress induced by H_2O_2/MPO system: cytotoxicity was assessed by the release of preincorporated ^{51}Cr. Propofol inhibited the CL produced by stimulated PMN in a dose dependent manner (until 5×10^{-5} M, a clinically relevant concentration), while Diprivan® and IL were not dose-dependent inhibitors. The CL produced by endothelial cells was dose-dependently inhibited by Diprivan® and PPF, and weakly by IL (not dose-dependent). In cell free systems, dose-dependent inhibitions were obtained for the three products with a lower effect for IL. Diprivan® efficaciously protected endothelial cells submitted to an oxidant stress, while IL was ineffective. By HPLC, we demonstrated that PPF was not incorporated into the cells. The drug thus acted by scavenging the active oxygen species released in the extracellular medium. IL acted in the same manner, but was a less powerful antioxidant.

Key words: Active oxygen species, Propofol, Diprivan®, Intralipid, Polymorphonuclear neutrophils, Endothelial cells

Introduction

Active oxygen species (AOS) are involved in tissue injury associated with many acute inflammatory processes including sepsis, acute lung injury and severe trauma.[1,2] The liposoluble anaesthetic drug, propofol (2,6-diisopropylphenol) (PPF), shares a similar structure with phenolic antioxidants like the endogenous α-tocopherol (vitamin E) which has been shown to protect cellular membranes against lipoperoxidation processes induced by AOS.[3] PPF has been demonstrated to inhibit the *in vitro* lipoperoxidation of microsomes and mitochondria isolated from rat liver, at clinically relevant doses,[4,5] and in a similar way as α-tocopherol.[6] In addition, it has been reported that rats receiving PPF have hepatic microsomes more resistant to lipoperoxidation.[7] By its antioxidant effects, PPF stabilizes the rat liver mitochondrial membranes exposed to an oxidative stress, inhibiting the Ca^{2+} induced permeabilization of mitochondria.[8] PPF has also been demonstrated to protect erythrocyte membranes against free radicals produced by thermic decomposition of an azo-compound[9] and to inhibit hyaluronan depolymerization by AOS produced by xanthine oxidase activity.[10] PPF could also attenuate ischaemia reperfusion injury.[11,12] Finally, we have shown that PPF could inhibit the peroxidation of a lipidic emulsion induced by ferryl, oxoferryl or hydroxyl radicals, and that its inhibiting effect was as efficient as that of α-tocopherol.[13]

The effects of clinically relevant doses of Diprivan®, the commercial form of PPF, on AOS produced by stimulated polymorphonuclear neutrophils (PMN), have been investigated by assessing different cell functions such as deformability, chemotactism, respiratory burst, phagocytosis and bactericidal activity. These studies yielded contradictory results. Diprivan® has been reported to inhibit the cell mobility,[14] the PMN chemotactically induced polarization,[15,16] the PMN hydrogen peroxide (H_2O_2) production,[17,18] as well as the cell capacity to phagocyte and kill bacteria such as *Staphylococcus aureus* and *Escherichia coli*.[19] Chemiluminescence studies performed on isolated PMN stimulated by N-formyl methionyl phenylalanine (FMLP) or by zymosan reported that Diprivan® inhibited the production of the AOS.[20–23]

In contrast, other studies have shown that Diprivan® did not modify the respiratory burst and the phagocytosis activity of PMN.[24,25]

In fact, intralipid (IL), the solvent of PPF in Diprivan®, has been demonstrated to inhibit the mobility, the respiratory burst and the phagocytosis capacity of PMN.[14,18,19,26] Therefore, it may be important to assess separately the effects of Diprivan®, IL and PPF.

The present study was designed to investigate separately the effects of PPF (diluted in an appropriate medium that did not interfere with the action of PPF), Diprivan® and IL on the stimulation of PMN, and on the production of H_2O_2 by cultured endothelial cells, using luminol-enhanced chemiluminescence (CL).[27] Cell-free systems where CL resulted from sodium hypochlorite (NaOCl) or from the reaction of H_2O_2 with peroxidases were also studied. In addition, the effect of Diprivan® on cultured human endothelial cells submitted to an oxidant stress was also studied, to examine the potential interest of this agent in clinical situations characterized by an excessive PMN stimulation leading to an endothelium oxidative stress.[1,2] Using HPLC technique, we quantified the incorporation of the drug into the PMN or endothelial cells to establish the extra- or intracellular mechanism of action of PPF.

Methods

Materials

Sodium, potassium, ammonium, calcium and magnesium salts, glucose, H_2O_2, NaOCl, diethyleneglycolmonoethylether (carbitol), thymol, cyclohexane, acetonitrile, trifluoroacetic acid (HPLC grade), and the HPLC column (Nucleosil RP-18, 5 µm particle size, 125 × 5 mm) were purchased from Merck (Germany). 3-Aminophtalhydrazide (luminol), trypan blue, human serum, heparin, gelatine, phorbol-12-myristate-13-acetate (PMA) were from Sigma Aldrich (Belgium). Fetal calf serum, M199 Medium, antibiotics, culture dishes and Polymorphprep™ were from Gibco BRL (Belgium). Endothelial cell growth factor (ECGF), dispase, horseradish peroxidase (HRP) were from Boehringer-Mannheim (Belgium); ^{51}Chromium (^{51}Cr) (sodium chromate) was from Amersham (Belgium).

Propofol and Diprivan® were gifts from Zeneca (Belgium). Intralipid was from Kabi Pharmacia (Belgium). Human myeloperoxidase (MPO) was purified in our laboratory as previously described.[28]

Phosphate-buffered saline (PBS) was: Na_2HPO_4 1.15 g/l; KH_2PO_4 0.2 g/l; KCl 0.2 g/l; NaCl 8 g/l. Hanks' balanced salt solution (HBSS) was: Na_2HPO_4 0.48 g/l; KH_2PO_4 0.06 g/l; $NaHCO_3$ 0.35 g/l; KCl 0.4 g/l; NaCl 8 g/l; $MgCl_2.6H_2O$ 0.1 g/l; $MgSO_4.7H_2O$ 0.1 g/l; $CaCl_2.2H_2O$ 0.185 g/l; glucose 1 g/l.

Isolation of PMN

Human PMN were isolated from buffy coats of healthy donors (Blood Transfusion Centre, University Hospital of Liège) by density gradient centrifugation. Buffy coats were added with 1 volume of Polymorphprep™ and centrifuged at room temperature (500 × ***g***, 30 min). The supernatant was diluted with 0.5 volume of 0.9% NaCl and centrifuged (1000 g × ***g***, 20 min). The collected cells were washed with a hypotonic solution (155 mM NH_4Cl, 170 mM Tris-HCl, pH 7.4) to lyse remaining erythrocytes. After centrifugation (800 × ***g***, 15 min), the collected cells were washed and adjusted to 10×10^6 cells/ml in NaCl 0.9% (stock solution). Viable cells in this preparation, assessed by the exclusion of trypan blue, were higher than 98%.

Human endothelial cell culture

Endothelial cells were isolated from human umbilical vein (HUVEC) by dispase treatment according to Jaffe *et al.*[29] HUVEC were cultured on 0.2% gelatine coated dishes in M199 medium supplemented with 10% heat-inactivated fetal calf serum, 5% heat-inactivated human serum, penicillin (100 U/ml), streptomycin (100 µg/ml), heparin (90 µg/ml) and ECGF (20 µg/ml). The cells were used at passage 2.

Drug solutions

The commercial form of PPF (Diprivan® : PPF 10 mg/ml) is formulated in intralipid (IL), a lipid vehicle emulsion (10% soya bean emulsion, egg phosphatides and glycerol). Tests were performed separately with PPF, Diprivan® and IL. Diprivan® or equivalent volumes of IL were added to the reaction milieu and compared with results obtained in the absence of Diprivan® and IL (control value taken as 100%). Before addition to the test tube, pure PPF was dissolved in carbitol to obtain the appropriate concentrations. A volume of 5 µl of the drug solution was always added to a final volume of 0.5 ml of PBS. Control tests were performed with 5 µl of carbitol alone, and the resulting CL value was taken as 100% (control value).

Chemiluminescence assays

Luminol enhanced chemiluminescence (CL) was measured in a Bio-Orbit 1251 Luminometer. All the CL assays were performed in a final volume of 0.5 ml PBS. The CL values were recorded over time and computerized. Maximal light emission (peak CL) and total emitted light (area under the time curve) were considered. Results were expressed as the percentage inhibition of the control value.

PMN CL assay

CL was measured at 37°C in the presence of 5×10^5 PMN (50 µl of the stock solution), luminol (10^{-4} M)

and appropriate concentrations of Diprivan®, IL or PPF dissolved in carbitol. The reaction was started by addition of PMA (10^{-6} M). The CL was recorded for 10 min. The results represent means ± SD of five independent assays performed with PMN isolated from five different buffy coats.

HUVEC CL assay

Adherent HUVEC were washed three times with PBS and gently scraped with a rubber policeman into PBS. After centrifugation, the cells were suspended in PBS (5×10^6 cells/ml). The assay was performed at 37°C, in a final volume of 0.5 ml of PBS containing 100 µl of the cell suspension (final number of cells: 5×10^5), luminol (10^{-4} M) and horseradish peroxidase (HRP) (2.5 U/ml). The CL was measured for 20 min. The assays were repeated three times.

NaOCl CL assay

The assay was done in the presence of luminol (5×10^{-7} M). NaOCl (10^{-5} M final concentration) was injected and the peak value of CL was immediately recorded. Each assay was done in triplicate and the experiment was repeated three times (n=9).

H_2O_2/peroxidase CL assay

Two peroxidases were tested: MPO (1.4 µg/ml) and HRP (0.2 U/ml). The assay was performed in the presence of luminol (10^{-4} M). H_2O_2 at the final concentration of 10^{-6} M or 2×10^{-7} M for MPO or HRP respectively, was injected and the peak value of CL was recorded. Each assay was done in triplicate for MPO and nine times for HRP.

Cytotoxicity assay on HUVEC

Cytotoxicity was assessed by measuring the release of previously incorporated ^{51}Cr. Confluent HUVEC in six multiwell plates were labelled overnight by 10 µCi/ml ^{51}Cr added in the culture medium. HUVEC were washed in HBSS to remove unincorporated ^{51}Cr, and then incubated for 1 h in 1 ml of HBSS containing MPO (5 µg), with or without addition of Diprivan® or IL. H_2O_2 (10^{-4} M) was added to initiate the enzymatic activity of MPO. After a further 2 h incubation at 37°C, the supernatants were collected and the cells were washed three times with HBSS. Supernatant and washings were pooled and ^{51}Cr release was quantified by γ counting. Cells were lysed in NaOH (1 N) and the intracellular ^{51}Cr was counted. The percentage of ^{51}Cr release was calculated for each test condition. An index of cytotoxicity (IC) was calculated as described elsewhere.[28]

The protective effect of Diprivan® against the oxidative stress was calculated as follows

$$\%\,\text{protection} = 100 \times \left(1 - \frac{^{IC}\text{Diprivan®}}{^{IC}\text{stress}}\right)$$

where $IC_{Diprivan®}$ and IC_{stress} were respectively the IC obtained in the presence or in the absence of Diprivan®. The experiment was repeated with HUVEC isolated from two different donors (n=9).

HPLC analysis of PPF incorporation into the cells

PMN or HUVEC (5×10^5 cells) were incubated with Diprivan® or PPF (10^{-4} M or 10^{-3} M) for 1 h at 37°C in 1 ml of HBSS. After incubation, the supernatant and the cells were separated and PPF was extracted by cyclohexane after addition of thymol as internal standard.[30] The solvent was evaporated to dryness at ambient temperature under nitrogen. The residue was dissolved in HPLC mobile phase, acetonitrile/H_2O/trifluoroacetic acid (600:400:1 by vol.), and submitted to HPLC analysis on RP-18 column with UV detection. The experiment was repeated twice.

Statistical analysis

All the results were expressed in percentage of control and presented as mean values with the standard deviation. Statistical analysis was performed using the Student's t-test. $P < 0.05$ was considered statistically significant. Dose–response curves were fitted by regression models (Pearson's test) using PPF concentrations in logarithmic scale.

Results

Effect of Diprivan®, IL and PPF on the CL of activated PMN

The effects of different concentrations of IL and Diprivan® on the CL of activated PMN are shown in Table 1. The percentage of inhibition was variable, depending on the different preparations of PMN. However in all cases, IL alone yielded an inhibitory effect which did not differ significantly from the effect of Diprivan®.

Pure PPF inhibited the CL of PMN in a dose-dependent manner (Fig. 1), in the range of 5×10^{-5} M (37.3 ± 6.3 percentage of inhibition) to 10^{-3} M (93.5

Table 1. Effects of Diprivan® on the chemiluminescence (CL) of 10^{-6} M PMA stimulated PMN: comparison with the effects of intralipid (IL), the vehicle solution of PPF in Diprivan®

PMN preparation	% of inhibition of CL Diprivan®/IL			
	volume in µL			
	0.09	0.9	2.7	9
1	0/22	84/73	97/96	98/95
2	0/0	39/35	52/47	53/47
3	25/47	24/19	68/61	71/57
4	–	76/75	70/73	84/82

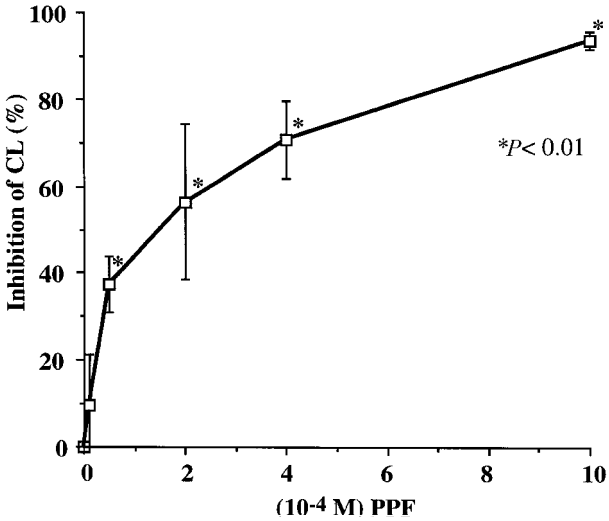

FIG. 1. Dose-dependent inhibition (±SD) of the CL of stimulated PMN by PPF (n=5).

± 2.1 percentage of inhibition). The concentration of 5×10^{-5} M propofol corresponds to a clinically relevant concentration. A significant linear correlation was obtained between the inhibition of CL and the PPF (logarithmic scale) concentrations (r^2=0.930).

Effects of Diprivan®, IL and PPF on the CL of HUVEC

IL and Diprivan® inhibited the HUVEC response, Diprivan® yelding a higher effect than IL (Fig. 2). For Diprivan®, inhibition was observed from 10^{-6} M (25.8 ± 6.8 percentage of inhibition) to 10^{-4} M (95.6 ± 1.1 percentage of inhibition). A saturation effect was obtained with IL: inhibition of CL rapidly reached a plateau at 30.9 ± 0.2 percentage of inhibition. Pure PPF inhibited the CL of HUVEC in a dose-dependent manner (Fig. 2) in the range of 2.5×10^{-6} M to 10^{-4} M (respectively 28.4 ± 5.8 and 96.0 ± 1.8 percentage of inhibition). For pure PPF and Diprivan® a linear correlation was found between inhibition of CL and the drug concentration (respectively r^2 = 0.943 and 0.955). The PPF concentrations required for CL inhibition of HUVEC were lower than for CL inhibition of PMN, but PMN produced more AOS than endothelial cells: the CL of PMN was 40 times that of HUVEC.

Effects of Diprivan®, IL and PPF on the CL of NaOCl

The CL resulting from NaOCl was inhibited by Diprivan® and IL, in a dose-dependent manner (linear correlations with respectively r^2 = 0.953 and 0.940) (Fig. 3) but Diprivan® yielded the most inhibiting effect. A significant inhibition (35.8 ± 1.0%) was already obtained with 3×10^{-6} M of Diprivan®, while IL was ineffective.

For pure PPF dissolved in carbitol the inhibition of CL was already significant for a PPF concentration of 5×10^{-6} M (25.9 ± 6.7 percentage of inhibition) (Fig. 3). A linear correlation was obtained between inhibition of CL and the PPF (logarithmic scale) concentrations (r^2= 0.914).

Effects of Diprivan®, IL and PPF on the CL of H_2O_2/peroxidase systems

H_2O_2/HRP

The effects of Diprivan®, IL and pure PPF on the CL of the H_2O_2/HRP system are presented in Fig. 4A. Inhibition of the CL was again observed with Diprivan® and IL with a more pronounced effect for Diprivan® (inhibition in the range of 10^{-6} M to 10^{-4} M of Diprivan® with respectively 20.0 ± 0.8 and 98.6 ± 0.1 percentage of inhibition). The inhibitiory effect of pure PPF dissolved in carbitol was also observed: 11.7 ± 0.3 percentage of inhibition for 3×10^{-6} M to 92.7 ± 0.3 percentage of inhibition for 3×10^{-5} M. Linear correlations were obtained between inhibition of CL

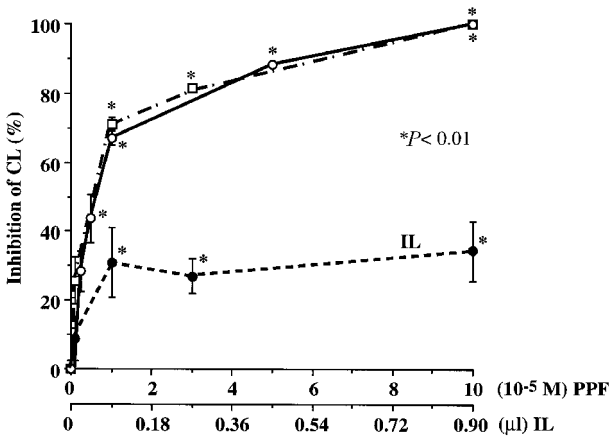

FIG. 2. Inhibition (±SD) of the CL of endothelial cells by pure PPF dissolved in carbitol (○——○). Diprivan® (□-·-□) or intralipid (IL) (●- - -●) (n=9).

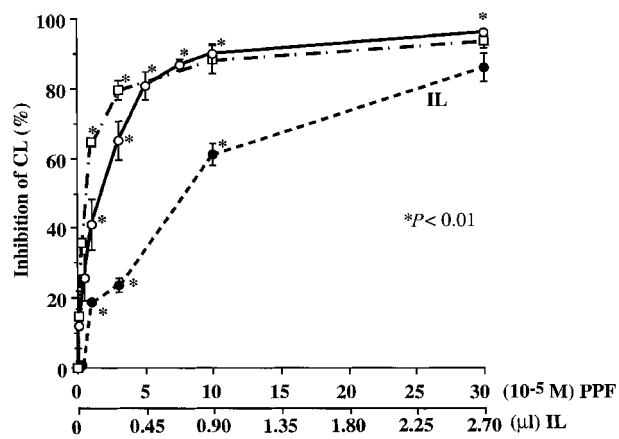

FIG. 3. Inhibition (±SD) of the CL of NaOCl by pure PPF dissolved in carbitol (○——○), Diprivan® (□-·-□) or intralipid (IL) (●- - -●) (n=9).

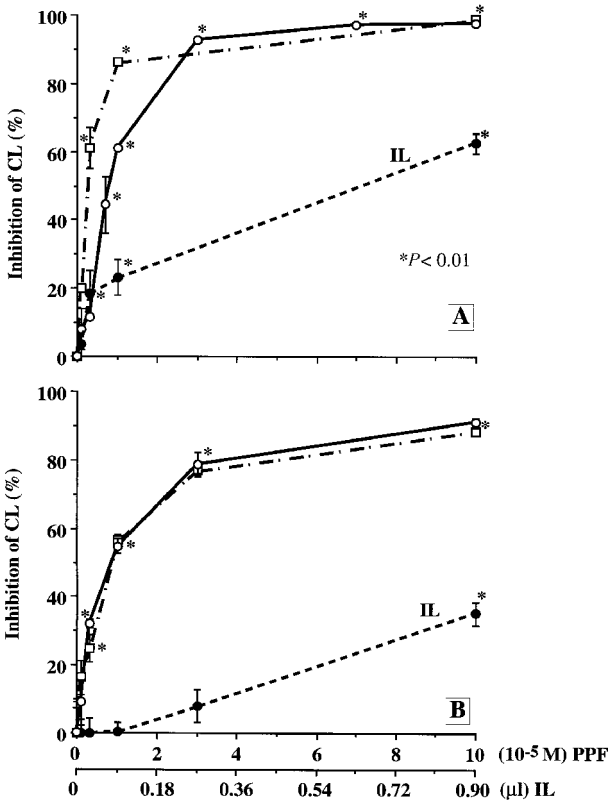

FIG. 4. Inhibition (±SD) of the CL of the H_2O_2/peroxidase system (above: H_2O_2/HRP; below: H_2O_2/MPO) by pure PPF dissolved in carbitol (○——○), Diprivan® (□—·—□) or intralipid (IL) (●– – –●) (n=9 for H_2O_2/HRP; n=3 for H_2O_2/MPO).

and the drug concentration (logarithmic scale) (r^2= 0.96, 0.98 and 0.92 respectively for Diprivan®, IL and pure PPF).

H_2O_2/MPO

The effects of Diprivan®, IL and pure PPF on the CL of H_2O_2/MPO system are presented in Fig. 4B. Linear dose-dependent inhibition of the CL was observed (r^2=0.96, 0.87 and 0.98 respectively for Diprivan®, IL and pure PPF). The inhibiting effect was already significant at 3×10^{-6} M PPF dissolved in carbitol (32.1 ± 1.2 percentage of inhibition) and 3×10^{-6} M Diprivan® (24.9 ± 3.9 percentage of inhibition), while IL was ineffective.

Effect of Diprivan® and IL on HUVEC submitted to MPO/H_2O_2/Cl^- oxidative stress

The MPO/H_2O_2/Cl^- system induced an oxidative stress on HUVEC (IC=32.8 ± 7.6%). Diprivan® exerted a protective, dose-dependent effect (r^2=0.783) on HUVEC against this oxidative stress, in the range of 0.3 to 3×10^{-4} M PPF concentrations (respectively 27.8 ± 6.3 and 97.6 ± 5.8 percentage of protection) (Fig. 5). No protection was observed with IL alone. Because of a cytotoxic effect of carbitol after 3 h incubation, PPF dissolved in carbitol was not tested in this experiment.

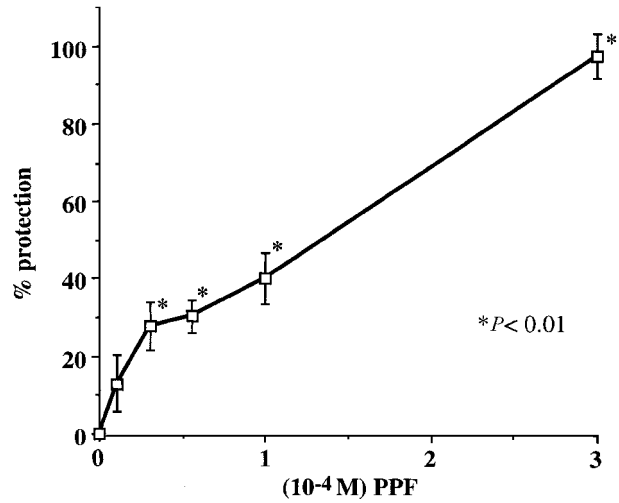

FIG. 5. Dose-dependent protection (±SD) of endothelial cells by Diprivan® against oxidant stress (n=9).

PPF incorporation into PMN and HUVEC

Small amounts of PPF were incorporated into PMN. PMN incubated with 10^{-3} M Diprivan® or PPF in carbitol incorporated respectively 2.5% and 5.1% of the drug; for 10^{-4} M Diprivan® or PPF in carbitol, we found respectively 0% or 2.2% of incorporation. No PPF incorporation was found into HUVEC.

Discussion

The present study was designed to investigate separately the effects of pure PPF, IL and Diprivan® on the production of AOS by PMN and endothelial cells, and on cultured human endothelial cells submitted to an oxidant stress. First, the study emphasized the dose-dependent inhibiting effect of PPF (diluted in carbitol) on the luminol-enhanced CL produced by stimulated neutrophils and endothelial cells. In addition, it showed the capacity of PPF and Diprivan® to inhibit the chemiluminescence of NaOCl and of the H_2O_2/peroxidase systems. These effects could be attributed to the non-hypnotic properties of Diprivan®. According to other results of this study, IL, the emulsified vehicle solution of PPF in Diprivan®, yielded a lower, but significant and dose-dependent inhibiting effect on the CL of HUVEC and cell-free systems. The HPLC study showed that PPF did not enter the cells. The CL inhibition could thus be attributed to a scavenging action on AOS, and not to an inhibition of the AOS production by the cells.

Considering the production of AOS by stimulated PMN, the inhibiting effects of Diprivan® were variable depending on cell batches and dose-dependent relationship could not be clearly established. The inhibiting effect of IL tested separately did not significantly differ from that of Diprivan®. This inhibitory effect of IL is quite difficult to explain. It might be due to the physical properties of the

emulsion producing a nephelometric effect in the reaction vessel during the CL measurement; however, this is unlikely as the volumes of IL used in our assays were too small to obtain this nephelometric effect. A chemical reaction of IL with the AOS is more likely: IL contains mainly triglycerides with saturated and unsaturated fatty acids. The unsaturated lipids could act as AOS scavengers. IL could also act directly on cell membranes, and induce structure alterations leading to a decrease of the AOS release in the extracellular medium with a decrease of the CL response. Inhibitory effects of IL on the functions (chemotaxis, phagocytosis, bactericidal activity, superoxide anion generation) of PMN were previously reported and often attributed to changes of the structure of the cell membranes.[14,15,18] Propofol alone (diluted in a non interfering solvant) had dose-dependent inhibiting effect on the CL response of PMN and endothelial cells, and was active at clinically relevant concentrations (range: 10^{-5} to 10^{-4} M).

These new results confirmed our first observations of an *in vitro* antilipoperoxidant effect of pure PPF[13] and were in agreement with the data collected from the literature, which demonstrated the *in vitro* antioxidant and antiradical properties of PPF in cell-free systems of erythrocyte membrane, microsome and mitochondria membrane lipoperoxidation.[4–9] The effects of PPF on PMN functions like chemotactism, respiratory burst and phagocytosis have been extensively investigated,[15–19] but few data using the chemiluminescence technique are available. Four studies[20–23] reported the inhibiting effect of Diprivan® on the CL produced by N-formyl-methionyl-leucyl-phenylalanine (FMLP) or zymosan-stimulated PMN and on FMLP stimulated PMN after priming with TNF-α. From these studies, it appeared that IL was less active than Diprivan®.[20–22] Regarding the different AOS producing systems in our study, the dose-dependent inhibiting effect of PPF dissolved in carbitol differed from that observed with Diprivan®. In the majority of the experimental conditions, the effects of Diprivan® at low concentrations reflected additive effects of IL and pure PPF. The percentage of inhibition of Diprivan® at 10 and 30 µM PPF on the CL of NaOCl was equivalent to the sum of inhibition values measured with IL and PPF dissolved in carbitol. In the same way, considering the H_2O_2/ HRP system, the CL inhibiting effect of 10 µM Diprivan® was the sum of the inhibition of IL and of PPF dissolved in carbitol. In contrast, the CL assays performed on stimulated endothelial cells did not yield similar results. In these conditions, the inhibiting effect of increasing concentrations of IL quickly reached a plateau. This plateau effect reflects a lesser scavenging activity of IL compared with PPF and a difference in AOS production by HUVEC and PMN. Hence, it is unlikely that HOCl could be produced by HUVEC given the absence of MPO into these cells.

The experimental conditions using different CL producing systems provide us with a partial understanding of the mechanism of action of pure PPF on the AOS. In fact, the CL of stimulated PMN reflects the global production of AOS including superoxide anion ($O_2^{\bullet}$) and its derivative hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) produced by the enzymatic activity of MPO, $NO^{\bullet}$, peroxynitrite (resulting from the reaction between $O_2^{\bullet}$ and $NO^{\bullet}$) and other excited oxygen species such as singlet oxygen and hydroxyl radical.[31] PPF may exert its inhibiting effect on CL of PMN at different levels. It has already been shown to neutralize hydroxyl radical.[13] From other studies, it appeared that PPF could affect the AOS produced by the activity of xanthine oxidase,[10] and restore the relaxing effect of aortic rings impaired by AOS.[32] Recently, PPF was demonstrated to be a scavenger of peroxynitrite.[33,34] The observations reported here indicate that PPF also may act both on HOCl and H_2O_2, or directly affect the activity of peroxidases. Its mechanisms of action on stimulated endothelial cells are still difficult to accurately define; indeed, these cells are able to produce $O_2^{\bullet}$ and H_2O_2 from intracellular xanthine oxidase, as well as peroxynitrite from $NO^{\bullet}$.

Finally, the inhibiting property of PPF might be considered as an unwanted side effect of the drug, since it could reduce the phagocytosis activity of PMN. However in the experimental conditions of the present study, the PMN stimulation by PMA resulted in a degranulation process and the release of active enzymes and AOS outside the cells, as demonstrated by the measurement of active granulocytic enzymes (MPO, elastase) in the reaction milieu. Should the scavenging activity of PPF be directed only against the excited oxygen species released outside the cells, PPF will be protective against the cellular and tissue destruction induced by an oxidative stress. Such a stress could be produced particularly by HOCl, a potent oxidant molecule capable of reaching areas quite distant from its production site. By chromatographic analysis (HPLC), we demonstrated that PPF does not enter the cell (neither PMN nor endothelial cells) and therefore does not act intracellularly. Moreover, we also demonstrated that Diprivan® protects endothelial cells against an oxidative stress while intralipid alone was without effect, and we recently reported an increased antioxidant capacity of plasma in patients anaesthetized with PPF.[35] These results could suggest a potential benefit of PPF in clinical situations characterized by an excessive PMN activation in the absence of cytotoxic compounds resulting from the reaction of this agent with AOS.

In conclusion, using the luminol-enhanced chemiluminescence technique, we demonstrated that the inhibiting property of PPF on the production of AOS in different experimental conditions is also shared

(but to a lesser degree) by the lipidic emulsion IL, which is the vehicle solution of PPF in Diprivan®. Both the active principle and the solvent have dose-dependent effects which are additive at low concentrations. Diprivan® was also protectvie for endothelial cells submitted to an oxidant stress. As PPF did not enter the cells, the drug would act by scavenging the active oxygen species released in the extracellular medium. IL would act in the same manner, but with a lower antioxidant capacity.

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