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Propofol protects cultured brain cells from iron ion-induced death: comparison with trolox

André Boland [a], Danielle Delapierre [a], Danielle Mossay [a], Pol Hans [b], Albert Dresse [a,*]

[a] *Laboratory of Pharmacology, Institute of Pathology B23, University of Liege, B-4000 Liege, Belgium*
[b] *Department of Anesthesia and Intensive Care Medicine, University Hospital of Liege, B-4000 Liege, Belgium*

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Abstract

The anesthetic propofol (PPF) has been shown to be an antioxidant in acellular experiments. This study was designed to assess the ability of PPF to protect primary-cultured brain cells against iron-mediated toxicity. A comparison with trolox (TX), a hydrosoluble vitamin E analogue, was performed. Rat cortical cells were exposed to 10 μM $FeSO_4$, PPF and/or TX. After a 4-h incubation, PPF and TX improved cell survival (lactate dehydrogenase measurements) in a concentration-dependent manner. The respective EC_{50s} of each substance were 4 and 4.6 μM. The maximal effect was obtained at a 25-μM concentration which is similar to concentrations of PPF used clinically. The combination of both drugs at certain concentrations showed a complete protection of the cells, a significant decrease in intracellular peroxide production (dichloro-fluorescein diacetate (DCF-DA) fluorescence, 4-h incubation), in lipoperoxidation (thiobarbituric acid reactive substances fluorescence, PPF 6.25 μM + TX 12.5 μM) and an additive protective effect. This was true after 4- and 16-h incubation. These data suggest that PPF is neuroprotective. Moreover, the combination with a vitamin E analogue confers long duration protection against oxidative stress.

Keywords: Oxidative stress; Free radical; Antioxidant; Cortical culture; Propofol; Vitamin E

1. Introduction

Increasing evidence suggests that oxidative stress induced by free radicals participates in tissue damage associated with acute pathological processes and in the pathophysiology of some neurodegenerative disorders such as amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (Olanow, 1993; Richardson, 1993; Smith et al., 1995; Williams, 1995). In these conditions, it is commonly believed that reactive oxygen species such as superoxide and hydrogen peroxide interact with iron ions, through the Fenton and Haber–Weiss reactions, to produce a final and very reactive product, the hydroxyl radical (°OH) (Halliwell and Gutteridge, 1992; Smith et al., 1995). Once produced in excess of the antioxidative capacities of the cell, these free radicals can alter many cell constituents and particularly polyunsaturated fatty acids of cell membranes. The oxidation of fatty acids into lipid peroxides results in a modification of the physico-chemical characteristics of membranes, such as an increased permeability to Ca^{2+}, leading to further production of free radicals, loss of cell integrity, apoptosis or cell necrosis (Olanow, 1993; Mattson, 1998). The brain appears to be particularly prone to damage by free radicals for many reasons, including the presence of large amounts of polyunsaturated fatty acids in cell membranes, a poor system of antioxidant defense and a high iron content. Indeed, ferrous iron is normally present at micromolar (1–3.5 μM) concentrations in the cerebrospinal fluid and this concentration is increased in certain neurological diseases (Halliwell and Gutteridge, 1992).

Vitamin E is one of the most potent endogenous antioxidants and has been reported to exert protective effects in experimental models of central nervous system traumatic injury and neuronal anoxia involving free radical formation (Weir et al., 1989). Propofol (PPF, 2,6-diisopropylphenol) is a hypnotic intravenous agent with antioxidant properties. It is chemically related to vitamin E and has been shown to inhibit lipid peroxidation in vitro and in vivo (Musacchio et al., 1991; Eriksson et al., 1992; Murphy et al., 1993; Aarts et al., 1995; Hans et al., 1996).

* Corresponding author. Tel.: +32-4-366-25-25; fax: +32-4-366-25-23.
E-mail address: dresse@pharmaco.medn.ulg.ac.be (A. Dresse).

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Therefore, the present study was designed to determine the effect of PPF on cultured cortical cells submitted to an oxidative iron-mediated stress. We compared the effects of PPF with trolox (TX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble derivative of vitamin E, known to protect various neuronal cells against oxidative stress (Chow et al., 1994; Boland and Dresse, 1995). The effect of combining the two drugs was also investigated using the same experimental model. The investigations were performed to understand both short- and long-term protection. Evaluation of cell protection was done by measuring cell death, intracellular peroxide production and lipid peroxidation.

2. Material and methods

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, Publication No (NIH) 85-23, revised 1985.

2.1. Mixed rat cortical cultures

Mixed primary rat cortical cultures were prepared from fetal Wistar rats (embryonic day E17–E19). After appropriate dissection, enzymatic dissociation was obtained by incubating cortices in 0.25% trypsin, phosphate buffered saline for 22 min, at 37°C. The reaction was stopped by adding fetal calf serum. The fragments were carefully washed using modified Eagle's medium (MEM, GIBCO-BRL, Cat. No. 11700-077) supplemented with 16 mM KCl, 26 mM sodium bicarbonate, 55 mM glucose, 1 mM L-glutamine and 1 mM pyruvate, and then mechanically dissociated. Then, these cells were plated in precoated dishes or multiwell plates (0.1 mg/ml poly-L-ornithin in borate buffer, 1 h at room temperature). For the thiobarbituric acid reactive substances assay, cells were plated in 50-mm dishes (Falcon, 3002) containing 2 ml MEM supplemented with 10% fetal calf serum at a density of 1 500 000 cells per dish. For 2,7-dichloro-fluorescein diacetate (DCF-DA) assays, cells were plated at a density of 150 000 cells/well (24-well plate, Falcon, 3047) containing 500 μl MEM–fetal calf serum.

Cell cultures were maintained at 37°C in a 95% air/5% CO_2 humidified atmosphere. After 4–6 h, the medium was renewed. The cells were then maintained for 7–10 days without any renewing of the culture medium. The same conditions were used for all experiments.

2.2. Antioxidant agents and iron exposure

Exposure to the protective agent and iron was performed in three steps: 7–10 days old cell cultures were washed three times with Locke's solution (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM HEPES and 10 mM glucose, pH 7.2, supplemented with 10 mg/l gentamycin) (Mattson et al., 1995; Blanc et al., 1997b). In the second step, the cells were exposed to the anti-oxidative substances for a 30-min preincubation. Finally, after the preincubation, 10 μM $FeSO_4$ (from a 400 μM stock solution prepared extemporaneously in bidistillated sterile water), was added and incubation was prolonged for either 4 (Goodman and Mattson, 1996) or 16 h.

2.3. Assessment of cell death

Cell death was assessed by the usual lactate dehydrogenase (E.C.1.1.1.27) activity assay which measures the quantity of lactate dehydrogenase released by dying cells (Blanc et al., 1997b).

After iron exposure, the extracellular medium was removed to perform the assay. Total releasable lactate dehydrogenase was obtained by submitting the cells to a freeze–thaw cycle. Phosphate buffer (2.5 ml) containing 0.1 mg/ml sodium pyruvate and 0.2 mg/ml β-nicotinamide adenine dinucleotide was added to 250 μl of culture medium sample. The absorbance decrease at 340 nm was then immediately determined for 6 min (Cecil Spectrophotometer, serial 2). Values were expressed as percentages of the total releasable lactate dehydrogenase.

2.4. Measurement of intracellular peroxides

The level of intracellular peroxides was quantified by fluorescence with 2,7-DCF-DA (molecular probe).

Cells were loaded with DCF-DA by incubating them for 50 min in presence of 100 μM DCF-DA. At the end of the incubation period, cells were washed once and the relative levels of fluorescence were quantified using a fluorescence plate reader (485-nm excitation and 535-nm emission, Victor Multilabel Counter, Wallach) (Blanc et al., 1997a). Values were expressed as percentage of fluorescence in control cultures.

2.5. Measurement of lipid peroxidation

The fluorescence of thiobarbituric acid reactive substances was used as a measure of the membrane lipoperoxidation. This test estimates the level of malonyl dialdehyde precursors, including hydroperoxides and endoperoxides (Blanc et al., 1997a).

After experimental treatment in 50-mm dishes, cells were washed twice with ice-cold phosphate buffered saline supplemented with 0.5 mM dithiotheitol. Cells were scraped into 250 μl of phosphate buffered saline–dithiotheitol mix and the samples were sonicated; aliquots were removed for protein determination. Trichloroacetic acid was added at a final concentration of 5%. Then, the thiobarbituric acid reactive substances reagent (four times concentrated solution containing 0.325% (w/v) 2-thiobarbituric acid in 50% glacial acetic acid) was added. The solution was incubated at 95°C for 30 min After cooling, 500 μl isobutanol were added, the samples were vigorously mixed and centrifuged at 1800 rpm for 10 min The

fluorescence of 200-μl aliquots of the upper organic phase was quantified using a Victor Multilabel Counter plate reader (485-nm excitation and 535-nm emission, Wallach). Values of thiobarbituric acid reactive substance fluorescence were expressed as the percentage of the level in vehicle-treated control cultures.

2.6. Quantitative analysis

Results are expressed as mean ± SEM.

In order to quantify protection afforded by the drugs, results were expressed as percent of total releasable lactate dehydrogenase activity. The concentration that produced 50% of the maximal effect (EC_{50}), was determined after curve fitting of the transformed data.

Data were analyzed using Student *t* test or analysis of variance (ANOVA).

3. Results

3.1. PPF and TX decrease oxidative stress-induced cortical cell death in a concentration-dependent manner

3.1.1. PPF

The addition of PPF to the medium, at concentrations ranging from 1.56 to 50 μM, improved the survival of cortical cells exposed to a 4-h 10 μM Fe^{2+}-induced oxidative stress. The protection increased in a concentration-dependent manner from 1.56 μM (69 ± 3% of total releasable lactate dehydrogenase) to reach a plateau corresponding to a complete cell survival at a PPF concentration of 25 μM (8 ± 1% of total releasable lactate dehydrogenase on the mean) (Fig. 1). After transformation of these data into percentage of the maximal effect and curve fitting, the evaluated EC_{50} of PPF was 4 μM.

3.1.2. TX

The addition of TX, at the same concentrations and for the same incubation duration as PPF, also produced a cell survival effect against Fe^{2+}-induced toxicity (Fig. 1). At the lowest concentrations of 1.56 μM, the protective effect of TX (61 ± 1% of total releasable lactate dehydrogenase) was not significantly different from PPF (69 ± 3% at the same concentration) ($P > 0.05$). The protective effect increased in a concentration-dependent manner from 1.56 μM to reach a maximum at 25 μM (6 ± 1% of total releasable lactate dehydrogenase). At the 12.5-μM concentration, both agents exerted a similar and marked protective effect (86% protection on average). As in the case of PPF, the protective effect obtained with 25 μM and higher, of TX, was maximal. The calculated EC_{50} of TX was 4.6 μM.

3.2. The combination of PPF and TX increases the cell survival against iron-induced oxidative stress

As described above, both TX and PPF had a protective effect. A complete cell survival however was only ob-

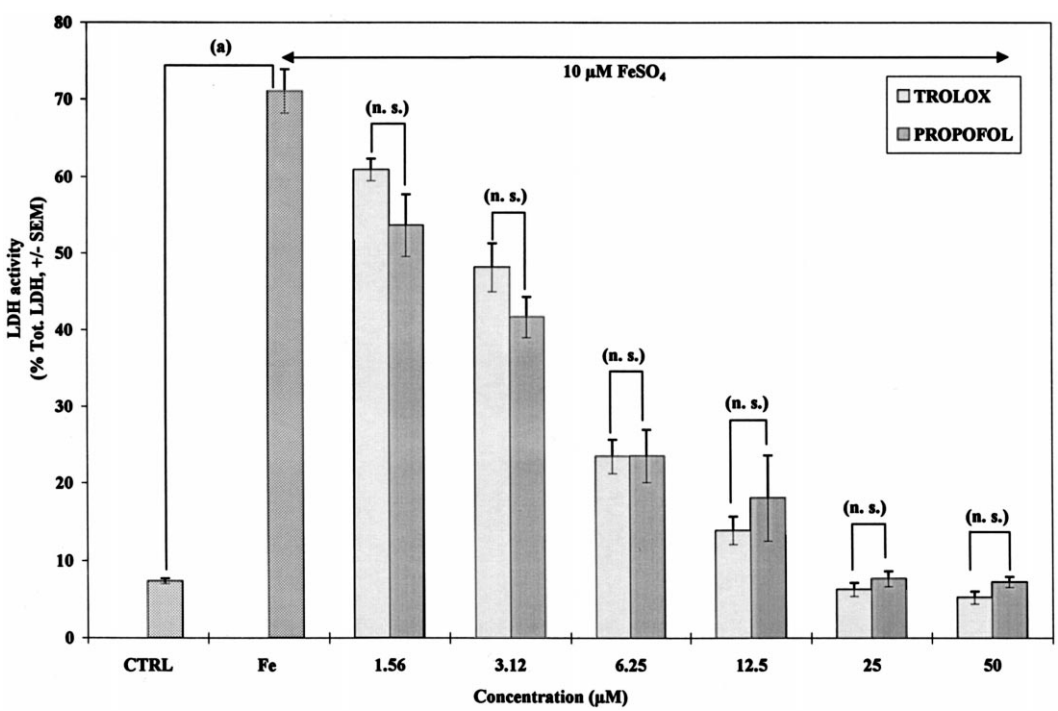

Fig. 1. Lactate dehydrogenase activity measurement of cortical cells exposed to 10 μM $FeSO_4$ and 1.56–50 μM of either PPF (hatched bars) or TX (dotted bars) during 4 h. Before adding iron, cells were pretreated for 30 min with either PPF or TX. The presence of iron alone induced a significant increase in cell death ($P < 0.0001$). The adjunction of antioxidant substances improved cell survival in a concentration dependent manner. Experiments were performed on, at least, three different cultures, (a) $P < 0.0001$; (n.s.) non significant difference.

tained at high concentration. We therefore wished to investigate if a combination of the two antioxidants would be more efficient. We assessed the effect of a combination of PPF and TX at the concentrations of 6.25 μM PPF (a concentration close to the measured EC_{50}) and 6.25 μM TX. A comparison with the effect obtained with each drug used individually was performed.

After 4 h of incubation, the combination of both PPF and TX at a 6.25 μM concentration conferred a marked protection of cortical cells. The corresponding lactate dehydrogenase release was 11 ± 1% of total lactate dehydrogenase, a value close to the control but significantly different (7 ± 0.3% total lactate dehydrogenase, $P = 0.0001$) (Table 1).

In order to compare the results obtained with those of the drugs used individually, we considered that the concentration of the combination was twice that of each drug alone. The lactate dehydrogenase value obtained with the combination of both 6.25 μM of PPF and 6.25 μM TX was not significantly different from that obtained with either 12.5 μM PPF or 12.5 μM TX ($P > 0.05$). These data shows that the effect of PPF and TX is simply additive, without synergy.

With the aim of obtaining a complete cell viability, we combined both substances and increased either PPF or TX concentrations. A 96% cell survival was obtained when the combination of 6.25 μM PPF + 12.5 μM TX was used on cells exposed to 10 μM Fe^{2+} during 4 h (this result was still significantly different from control ($P < 0.0001$)) (Table 1). The combination of both PPF and TX at a 12.5-μM concentration produced a complete protection after a 4-h iron-induced oxidative stress ($P > 0.05$ compared to control or 25 μM PPF or TX alone) (Table 1). These data confirmed the additive effect of both substances.

3.3. PPF and / or TX decrease Fe^{2+}-induced production of intracellular peroxide

To assess if PPF and/or TX were able to modify the production of intracellular free radicals, we measured the DCF-DA fluorescence induced by 10 μM iron in the presence of these protective substances.

Iron alone induced an important increase in DCF-DA fluorescence after a 4-h incubation (2.55 times the control value, Table 1). This observation confirmed that iron generates toxic intracellular peroxides.

Application of 6.25 μM PPF or TX induced a decrease of 48 and 63%, respectively in the DCF-DA fluorescence ($P < 0.0001$). A higher concentrations of 12.5 μM allowed an almost complete disappearance of intracellular peroxides (97% and 88% reduction by PPF and TX, respectively). The combination of PPF and TX both at a 6.25 μM concentration induced a decrease in DCF-DA fluorescence comparable to the one obtained when PPF or TX were used alone at 12.5-μM concentration. This confirmed their additive effect as described in Section 3.2.

3.4. PPF and / or TX decrease lipid peroxidation induced by Fe^{2+}

In order to determine if PPF and TX also caused a decrease in the $FeSO_4$-induced lipoperoxidation, we performed thiobarbituric acid reactive substances assays on cortical cells exposed to iron and antioxidative substances.

As shown in the Table 1, PPF and TX partially decreased the lipid peroxidation induced by 10 μM iron during 4 h. The comparison of the effects of PPF and TX shown that the 6.25 μM PPF concentration was more potent in reducing lipoperoxidation than the same TX concentration ($P < 0.05$). A higher TX concentration of

Table 1
Lactate dehydrogenase activity assessment, intracellular peroxides production measurement using the DCF-DA fluorescent probe and thiobarbituric acid reactive substances production estimation of cultured cortical cells submitted to 10 μM Fe SO_4 and a combination of PPF and TX during 4 h

A	B	C	D
Control	7.3 ± 0.3[c] (36)	1.00 ± 0.03[c] (13)	1.00 ± 0.03[c] (20)
Fe (10)	70.4 ± 2.7 (32)	2.55 ± 0.15 (10)	2.42 ± 0.06 (22)
PPF (6.25) + Fe (10)	23.4 ± 3.5[c] (16)	1.81 ± 0.11[b] (11)	1.67 ± 0.09[c] (18)
PPF (12.5) + Fe (10)	18.1 ± 4.2[c] (7)	1.05 ± 0.17[c] (3)	/
TX (6.25) + Fe (10)	23.5 ± 2.3[c] (17)	1.57 ± 0.18[b] (11)	2.13 ± 0.12[a] (10)
TX (12.5) + Fe (10)	13.9 ± 1.8[c] (20)	1.18 ± 0.06[c] (11)	1.89 ± 0.09[c] (14)
PPF (6.25) + TX (6.25) + Fe (10)	11.3 ± 1.2[c,d] (11)	1.20 ± 0.08[c] (11)	1.45 ± 0.07[c] (8)
PPF (6.25) + TX (12.5) + Fe (10)	10.0 ± 0.5[c,d] (17)	1.08 ± 0.14[c](11)	1.50 ± 0.13[b] (8)
PPF (12.5) + TX (12.5) + Fe (10)	7.2 ± 0.4[c,e](3)	0.64 ± 0.04[c] (3)	/

(A) Treatments (concentrations in μM); (B) lactate dehydrogenase activity (%total lactate dehydrogenase ± SEM)$^{(P)}$ (n); (C) DCF-DA fluorescence (values compared to control ± SEM)$^{(P)}$ (n); (D) thiobarbituric acid reactive substances fluorescence (values compared to control ± SEM)$^{(P)}$ (n).
The effect of various concentrations of both drugs are shown. Compared to iron-treated cells, both PPF and TX contributed to decrease significantly the cell death and the intracellular peroxide production. Both PPF and TX contributed to decrease partially, but significantly, the lipid peroxidation induced by 10 μM iron. Combination of PPF and TX did not ameliorate the protection afforded with PPF alone.

[c] $P < 0.001$ (compared to iron-treated cells).
[b] $P < 0.01$ (compared to iron-treated cells).
[a] $P < 0.05$ (compared to iron-treated cells).
[d] $P < 0.001$ (compared to control cells).
[e] Non-significant difference (compared to control cells; see text for further explanations).

12.5 μM did not significantly decrease the level of peroxidation compared to 6.25 μM TX ($P > 0.05$). The combination of 6.25 μM PPF and TX each, reduced lipid peroxidation. This reduction however was not greater than that obtained by PPF alone ($P > 0.05$). This indicates that PPF is more potent in limiting lipoperoxidation induced by oxidative stress than TX. This observation was further established with the use of 12.5 μM TX combined with 6.25 μM PPF. The reduction of the lipoperoxidation seen with this combination was not significantly different compared to the reduction with 6.25 μM PPF alone.

3.5. Long duration protection

In order to assess if the protection conferred was effective for a longer time, we exposed the cultures to the same oxidative stress and PPF and/or TX for the duration of 16 h. At the end of the incubation, lactate dehydrogenase, and thiobarbituric acid reactive substances assays were performed.

For this duration, we observed that 6.25 μM PPF concentration was not able to protect cells from an iron-induced death. The corresponding lactate dehydrogenase measurement was 89 ± 3% of total releasable lactate dehydrogenase (Table 2), a value corresponding to 2.8 times the one obtained after a 4-h incubation. On the other hand, a 12.5 μM TX concentration gave a significantly better cell survival compared to 6.25 μM PPF ($P < 0.0001$): the value of lactate dehydrogenase was almost two times less than the value obtained with 6.25 μM PPF alone (Table 2).

Table 2
Effect of the combination of PPF and TX on cell mortality and lipid peroxidation of cultured cortical cells submitted to 10 μM Fe SO_4 during 16 h

A	B	C
Control	23.61 ± 2.18[b] (15)	1.00 ± 0.06[b] (14)
Fe (10)	107.45 ± 4.29 (18)	2.67 ± 0.14 (16)
PPF (6.25) + Fe (10)	88.61 ± 2.92[a] (12)	2.17 ± 0.08[b] 12)
PPF (12.5) + Fe (10)	69.99 ± 3.30[b] (13)	1.99 ± 0.03[b] (12)
TX (12.5) + Fe (10)	49.25 ± 4.20[b] (12)	/
PPF (6.25) + TX (12.5) + Fe (10)	40.82 ± 1.83[b] (8)	2.02 ± 0.16[b] (12)
PPF (12.5) + TX (12.5) + Fe (10)	28.51 ± 0.64[b] (11)	/

(A) Treatments (concentrations in μM); (B) lactate dehydrogenase activity (%total lactate dehydrogenase ± SEM)$^{(P)}$ (n); (C) thiobarbituric acid reactive substances fluorescence (values compared to control ± SEM)$^{(P)}$ (n).

In order to know if the PPF or TX protection can be prolonged, we performed lactate dehydrogenase activity assessment and thiobarbituric acid reactive substances production measurements on cultured cortical cells submitted to 10 μM Fe SO_4 and a combination of PPF and TX during 16 h. Combination of PPF and TX allows to obtain a better protection of the cells. Moreover, PPF and TX contributed to decrease partially, but significantly, ($P < 0.001$) the lipid peroxidation induced by 10 μM iron, 12.5 μM PPF, gave the maximal effect.

[b] $P < 0.001$ (compared to iron-treated cells).

[a] $P < 0.01$ (compared to iron-treated cells).

The combination of both substances (6.25 μM PPF and 12.5 μM TX) increased slightly, but not significantly, the protective effect of 12.5 μM TX alone ($p > 0.05$) (Table 2).

To test if it is possible to obtain a full long-term survival of the cells, we looked at the protective effect of higher concentrations of both PPF and TX. For this purpose, we used 12.5 μM PPF and/or TX and estimated the lactate dehydrogenase release after a 16-h incubation. In these conditions, we observed that 12.5 μM PPF partially helps (70 ± 5% total lactate dehydrogenase) cortical cells to survive to a 10 μM $FeSO_4$-induced oxidative stress (Table 2). TX showed a significantly better protective effect than PPF at the same concentration ($P < 0.005$). The lactate dehydrogenase value obtained was 49 ± 4% of total lactate dehydrogenase. This protective effect is not significantly different from the one obtained for the combination of 6.25 μM PPF + 12.5 μM TX ($P = 0.14$). PPF alone therefore does not confer a good long duration survival. The use of a combination of PPF and TX at the 12.5-μM concentration each gave a full long-term protection of cortical cells exposed to 10 μM iron during 16 h. The lactate dehydrogenase value observed was 28 ± 1% of total lactate dehydrogenase. This value was not significantly different from the corresponding control (24 ± 3% of total lactate dehydrogenase, $P = 0.23$). This data highlights the need to use a combination of both PPF and TX to obtain a good survival of the cells during a prolonged duration.

The thiobarbituric acid reactive substances tests performed in the same conditions showed that PPF and TX were able to significantly decrease the level of lipoperoxidation products. The maximal effect was obtained with 12.5 μM TX alone (Table 2).

In these conditions, it was not possible to maintain the values close to the one of the control cells, neither for lactate dehydrogenase nor for thiobarbituric acid reactive substances.

Because of the long-term protection obtained with the combination of both 12.5-μM concentration PPF and TX, we also measured the DCF-DA fluorescence in these conditions after a 4-h incubation. The DCF-DA value obtained was significantly inferior ($P < 0.001$, Table 1) to the one of control cells and correspond to a 36% decrease of the fluorescence. The protection obtained at these higher concentrations of PPF and TX combined was therefore better than that obtained at 6.25 μM each, even in the short-term.

4. Discussion

Addition of $FeSO_4$ has been used by several authors (Eriksson et al., 1992; Murphy et al., 1993; Zhang et al., 1993; Chow et al., 1994; Goodman and Mattson, 1996) to induce neuronal cell damage. The damage induced by this

procedure appears to be mediated by free radical species such as the hydroxyl and the ferryl radicals (Hans et al., 1996). As mentioned in our introduction, this model might be relevant to neurodegeneration, therefore, according to many authors we used a 10-μM $FeSO_4$ concentration as an initiator of oxidative stress (Goodman and Mattson, 1996; Furukawa et al., 1997). This experimental condition triggers lipid peroxidation and/or generates °OH in cortical cell cultures.

In the short-term (4-h incubation), the improvement of cell viability by PPF was maximal at a 25-μM concentration (100% cell protection) but lower concentrations already show a protective effect against oxidative stress. The estimated PPF EC_{50} was 4 μM. Similar concentration (25 μM) are used clinically to provide sedation or anesthesia. Indeed, a peak plasma concentration of PPF of 40–60 μM is observed during the induction of anesthesia and the usual blood PPF concentrations required to maintain anesthesia during surgery range between 11 and 28 μM (Murphy et al., 1992; Aarts et al., 1995). Moreover, it has been reported that the blood-brain equilibration half time is 2.9 min (Kanto and Gepts, 1989). Therefore, PPF concentrations in the brain tissue may be close to its concentration in the plasma. Taken together, this data suggests that the concentration of PPF used during anesthesia may be neuroprotective. The free radical scavenging properties of PPF at anesthetic concentrations have already been demonstrated by other authors in in vitro and in vivo experiments (Musacchio et al., 1991; Murphy et al. 1993; Aarts et al. 1995; Hans et al. 1996).

It is to be noted that, for the TX (EC_{50} = 4.6 μM) the maximal cell survival is reached at 25 μM (100% cell protection). The TX concentrations used in our experiments are similar to those used by Chow et al. (1994) who examined the protective effect of TX on cortical neurons submitted to iron exposure (50 μM Fe^{2+} and 50 μM Fe^{3+}) (Chow et al., 1994). However, in this study, the authors only obtained a complete protection of cells with a much higher concentration of TX (100 μM). This may be explained by the fact that their experimental conditions were different from ours: the iron (used at non-physiological concentrations) and TX exposure were done in Eagle's MEM augmented with Earle's salts and 25 mM glucose bubbled with CO_2 to buffer the medium to pH 7.4 at room temperature. Afterwards, cultures were returned to the humidified 37°C, 5% CO_2 incubator for 24 h.

Beside the data concerning cell survival in vitro, we also demonstrate that PPF and TX can decrease the production of intracellular peroxides (DCF-DA experiments, Tables 1 and 2) and lipid-peroxidation (thiobarbituric acid reactive substance experiments, Table 2). Such a modification in lipoperoxidation has already been shown by many authors on cell-free systems (microsomes, synaptosomes...) (Musacchio et al., 1991; Eriksson et al., 1992; Green et al., 1994; Hans et al., 1996) using different types of oxidative stress.

To assess if a long duration protective effect of PPF and TX exists, we tested these substances, using the same oxidative stress, during 16 h. In these conditions (neither PPF nor TX were removed during the 16-h. incubation), we demonstrate that low concentrations of PPF (6.25 and 12.5 μM) cannot confer a long duration protection (Table 2) as well as the same concentrations of TX. In fact, in vivo, the PPF-blood concentration decreases quickly by rapid distribution, redistribution and metabolism (Kanto and Gepts, 1989). Therefore, supplementing PPF with a substance having anti-oxidative properties (TX or the natural liposoluble compound, vitamin E) might help brain cells to survive better when they are submitted to an ischemic stroke or a traumatic brain injury. Moreover, our study confirms the hypothesis of Hans et al. (1996) that PPF and TX have an additive effect (Table 1). We demonstrate that a combination of TX and PPF in the case of exposure to oxidative stress provides both long- and short-term cell protection.

The protective effect of these drugs might be explained by their mechanism of antioxidant activity (Murphy et al., 1992). Indeed, PPF is chemically similar to phenol-based free radical scavengers such as butylated hydroxytoluene and vitamin E (Murphy et al., 1992). Like phenol-based antioxidants, PPF has been demonstrated by Murphy and colleagues, using electron spin resonance spectroscopy, to scavenge free radicals by a process of hydrogen abstraction leading to the formation of a phenoxyl radical.

To complete our understanding of the mechanisms involved in PPF induced protection, it would be interesting to know whether PPF can improve mitochondrial functions, modify the protein function of cell membranes or protect against the toxicity of aldehydes produced during lipid-peroxidation. Such mechanisms involved in the protection afforded by vitamin E against oxidative stress or 4-hydroxy-nonenal toxicity are already known (Mattson, 1998). Therefore, an extension of the study to PPF protective mechanisms would be of interest.

We have shown therefore that PPF protects cortical cells against iron-mediated stress by improving cell viability and by decreasing intracellular peroxide production and lipid peroxidation. This protective effect is observed at concentrations similar to the one used clinically. It is likely to be related to its antioxidant properties, which are comparable to those of TX. These results provide an experimental support for investigating the potential benefit of using PPF as an anesthetic drug in patients suffering from diseases involving free radical reactions. It also shows the potential benefit of using PPF in combination with another anti-oxidant to increase the probability of survival in cells submitted to an oxidative stress.

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