Burn-induced oxidative stress is altered by a low zinc status: kinetic study in burned rats fed a low zinc diet

Richard Claeyssen[1,2], Maud Andriollo-Sanchez[1], Josiane Arnaud[2,3], Laurence Touvard[1], Antonia Alonso[1], Yves Chancerelle[1], Anne-Marie Roussel[2], Diane Agay[1].

[1] Centre de Recherches du Service de Santé des Armées, 24 Avenue des Maquis du Grésivaudan, BP87, 38702 La Tronche cedex, France.

[2] INSERM U884, Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Joseph Fourier, BP 53, 38041 Grenoble cedex 9, France.

[3] Département de Biologie Intégrée, CHU Grenoble, BP 217, 38043 Grenoble Cedex 9, France.

Keywords: Burn injury, zinc intakes, zinc status, oxidative stress, rat model.

Corresponding author and address for reprints:

Diane Agay
CRSSA
24 Avenue des Maquis du Grésivaudan
BP 87
38702 LA TRONCHE Cedex

Telephone number: +33 4 76 63 69 80
Fax number: +33 4 76 63 69 22
E-mail: diane.agay@crssa.net

Abstract

As an initial subdeficient status of zinc, considered as an essential antioxidant trace element, is frequent in burned patients, we aim to assess the effects of low zinc dietary intakes on burn induced oxidative stress, in an animal model. After eight weeks of conditioning diets containing 80 ppm (control group) or 10 ppm of zinc (depleted group), Wistar rats were 20% TBSA burned and sampled one to ten days after injury. Kinetic evolutions of zinc status, plasma oxidative stress parameters and antioxidant enzymes were also studied in blood and organs.

The zinc depleted diet induced, before injury, a significant decrease in zinc bone level and the increase of oxidative stress markers without stimulation of antioxidant enzyme activity.

After burn, more markedly in zinc depleted animals than in controls, zinc levels decreased in plasma and bone, while increasing in liver. The decrease of thiol groups and GSH / GSSG ratio and the depression of GPx activity in liver are also moderately emphasized.

Nevertheless, depleted zinc status could not be considered as determining for oxidative damages after burn injury. Further investigations must also be done to enlighten the mechanism of beneficial effects of zinc supplementation reported in burned patients.

Introduction

Zinc has a wide range of cellular processes including cell proliferation, immune function and defence against free radicals as well as genetic stability and function which are crucial to maintain in burn injury [1-3]. Mechanistically, several proteins involved in DNA damage signalling and repair, replicative enzymes (DNA and RNA polymerase) and transcription factors require zinc for proper function [4, 5]. Zinc is also thought to function as an antioxidant through a number of proposed mechanisms: cofactor of Cu-Zn superoxide dismutase, induction of synthesis of metallothioneins, stabilization of membrane lipids against oxidation, protection of thiol group against iron attacks released after burn injury [6-9].

After major burns, organisms suffer many unresolved metabolic, endocrine and immune alterations. Some of these changes can be ascribed to disturbance in trace element metabolism [10, 11]. A large involvement of these trace elements (zinc, copper, selenium, chromium) in the defence against thermal aggression has been demonstrated [12, 13]. Interventional studies in burn patients have demonstrated the beneficial effects of trace element supplementation in reducing the infectious complications, improving wound healing and shortening length of hospital stays [14]. Thus, zinc status could be determinant in the response against thermal injury. However, whereas low zinc intakes and subdeficient status are frequently observed in general population [15-17], few studies focused on the impact of a low zinc status in the response against burn injury, especially in counteracting the burn-induced oxidative stress.

Therefore, we aimed to evaluate, using an animal model of thermal injury moderately depleted in zinc, the effects of a zinc subdeficient status on burn-induced oxidative stress within the first days following the burn injury. A 20% total body surface area (TBSA) burn injury was applied on both rats receiving Zn adequate and Zn depleted diets. We followed the change of Zn status in plasma and tissues and different parameters in relation with burn-

induced oxidative stress, including thiobarbituric acid reactive species (TBARs) as markers of lipid peroxidation, sulfhydryl groups (SH) for protein oxidation, reduced and oxidized glutathione (GSH/GSSG) as endogenous antioxidant defences and glutathione peroxidase (GPx) and superoxide dismutase (SOD) as antioxidant enzymes.

Materials and Methods

Animal care

The study was conformed to the guidelines established by the National Institute of Health and the procedures were approved by the Animal Ethics Committee of the French Defence Medical Research Centre. All experiments were performed in accordance with the legislative standards prescribed by the French Ministries of Agriculture and Environment.

Three weeks old male Wistar rats were purchased from Charles River Laboratories (Saint Germain sur l'Arbresle, France). Animals were housed, first by four and then individually relating to their body weight, in thermoformed polystyrene cages and handled daily to minimize experimental stressors. The cages were located in a room with a 12h-12h light-dark schedule and a pressurized filter air barrier. The temperature was controlled to 21±1°C for the duration of the experiment with a relative humidity of 55%. Animals received sterilized food and water *ad libitum*. The litter was also sterilized to limit bacterial contamination risk for the immunodepressed burned animals. Water was demineralised to avoid zinc contamination.

Design of the study

Rats were conditioned with two different diets purchased from SAFE (Epinay sur Orge, France) with an adequate (80 ppm) or a depleted (10 pm) level of zinc (table 1). Just after

delivery, the eighty weaned rats were divided randomly into two series of 40 animals, the control group (C) receiving the 80 ppm Zn diet and the zinc depleted group (ZD) receiving the 10 ppm Zn diet. Each animal was weighed every week in order to follow their growth curve. The animals received their specific diets for 8 weeks.

After conditioning, each series (n=40) were randomly divided into eight groups (n=5) before being submitted to burn injury. For each group of diet (10 and 80 ppm Zn) non-burned rats (n=5) were used as initial values (T0) and the others animals were sacrificed one day (D1), two days (D2), three days (D3), four days (D4), five days (D5), eight days (D8) and ten days (D10) after thermal injury.

Experimental thermal injury

A non lethal third-degree thermal injury involving 20% TBSA was applied to the animals according to the method described by Walker and Mason [18]. Briefly, each rat was anaesthetized with halothane and its back was shaved before delimitation of the burn area. The anaesthetized rat was then fixed flat on its back on a perforated Plexiglas plate and the delimited skin surface was immersed throw the hole in a boiling water bath at 100°C during ten seconds. To stop the burn process, the rat was then immediately plunged into fresh water and dried with towel before awakening. As this burn procedure leads to a full thickness 3rd degree dermal wound, nervous terminations are histologically and functionally destroyed. The burn lesions appear painless and are also well tolerated by the rats. Therefore, no analgesic was further required for the burned animals.

Plasma and tissue preparation

- *Sample collection*

Animals were fasted overnight before sampling days. Under halothane anaesthesia, blood sample collection was performed after laparotomy by vena cava puncture using S-Monovette™ syringes (Sarstedt, Germany) with anticoagulant. Lithium heparinate trace element-free syringes were used for blood parameters determination. Immediately after blood collection, the rats were killed by section of heart vessels and liver, muscle, kidney and femur were removed, weighed, placed in mineral-free tubes and frozen in liquid nitrogen.

- *Plasma and whole blood samples*

For glutathione measurement, 200 µL of whole blood was mixed with 1.8 mL of metaphosphoric acid (MPA 6%) and after centrifugation (10 min; 3000g; 4°C), the supernatant was removed and stored at -80°C until analysis.

For glutathione peroxidase (GPx) determination, 25 µL of whole blood was mixed with 1 mL of RANSEL kit diluting agent (Randox Laboratories, Crumlin, UK) and stored at -80°C until analysis.

For other plasmatic parameters, after blood centrifugation (10 min, 3000 rpm, 4°C), the plasma was removed, dispatched in trace element free microtubes and stored at -80°C until analysis.

- *Tissue samples*

For trace element status determination, tissue samples were stored without any preparation in mineral-free tubes at -80°C. For oxidative status determination, liver, kidney and femoral quadriceps samples were extracted in lysis buffer (Tris-NaOH 10 mM, diethylenetriaminepentaacetic acid (DTPA) 1 mM, phenylmethanesulfonyl fluoride (PMSF) 1 mM, pH 7.4) with ratio “100 mg of tissue / 1 mL of buffer” and centrifuged for 10 min at 3000g at 4°C. First, 300 µL of supernatant were collected for thiobarbituric acid-reactive species (TBARs) measurement and 100 µL of supernatant was added to 100 µL of NaOH 1N for total proteins determination. After a second centrifugation (20 min; 12,000g; 4°C), 800 µL

of supernatant was collected for GPx activity determination. Samples were then stored until analysis at -80°C.

Biological parameters

- *Thiobarbituric acid-reactive species*

Lipid peroxidation was measured by TBARs production following a fluorimetric method described by Richard et al. [19]. The calibration curve was obtained from a stock solution of 20 $mmol.L^{-1}$ 1,1,3,3-tetraethoxypropane (TEP) in the range of 1 to 8 $mmol.L^{-1}$. Standards and samples were placed for one hour in a boiling water bath at 95°C in presence of a thiobarbituric acid (TBA) / perchloric acid ($HCLO_4$) mix (2/1 volume respectively) and then the reaction was stopped by cooling in ice. After extraction of the coloured malondialdehyde-TBA complex with butanol, the fluorescence was measured on a fluorescence spectrophotometer (Hitachi F4500, B. Braun ScienceTec, France) set at an excitation wavelength of 532 nm and an emission wavelength of 553 nm.

- *Plasma thiol groups*

Protein oxidation was determined as described by Faure et al. by plasma thiol (SH) groups determination [20]. The calibration was obtained from a stock solution of 1000 $\mu mol.L^{-1}$ N-acetyl cystein (NAC) in the range of 125 to 600 $\mu mol.L^{-1}$. Standards and plasma samples were measured by spectrophotometry at 415 nm (Hitachi 912, Roche Diagnostics, France) in the presence of 0.05 $mol.L^{-1}$ phosphate buffer, 1 $mmol.L^{-1}$ EDTA, pH 8 and 2.5 $mmol.L^{-1}$ bis-5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB).

Plasma Cholesterol

Plasma cholesterol was measured by spectrophotometry (Hitachi 912, Roche Diagnostics, France).

- *Total and soluble proteins*

Protein concentrations of tissue homogenates were determined by the Pierce method using bicinchoninic acid according to Smith et al. [21] with BCA^{TM} protein assay kit (Interchim, Montluçon, France) on an automatic analyzer (Hitachi 912, Roche Diagnostics, France).

- *Total and oxidized glutathione*

Total and oxidized glutathione (GSH & GSSG) were determined as described by Akerboom and Sies [22]. The reduction of DTNB into 5-thio-2-nitrobenzoate (TNB) was measured by spectrophotometry (Uvikon XS, Secomam, France) following absorption at 412 nm. Values were determined comparing the reduction rate against a standard curve of glutathione. Total glutathione levels (GSH) was measured in prepared whole blood samples (above-mentioned preparation) in the presence of 0.4 mmol.L^{-1} Morpholinopropanesulfonic acid (MOPS) / 2 mmol.L^{-1} EDTA buffer, 4.8 mmol.L^{-1} NADPH, 5 U.mL^{-1} glutathione reductase, 3.8 mol.L^{-1} DTNB. Oxidized glutathione (GSSG) were measured in the same conditions with a 1 h previously incubation of standards and samples with triethanolamin and 2-vinyl pyridine monomer.

- *Glutathione peroxidase*

GPx (EC 1.11.1.9) activity determination was evaluated according to Paglia and Valentine [23] with a RANSEL kit (Randox Laboratories, Crumlin, UK). Briefly, GPx catalyses the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP^{+}. The decrease in absorbance at 340 nm is measured.

- *Superoxide dismutase*

In plasma and tissues samples, superoxide dismutase (SOD) activity was performed using an adaptation of Woolliams et al. and Arthur et al. [24, 25]. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4iodophenyl)-3-(4-

nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50 % inhibition of the rate of reduction of INT under conditions of the assay.

- *Haemoglobin*

Haemoglobin was measured according to the International Committee for the Standardisation in Haematology (ICSH) [26] with Randox haemoglobin kit. Sample analyses were performed after quality controls with ABX Minotrol 8 -L (low), -N (normal) and -H (high) standards (Horiba ABX, Montpellier, France).

- *Zinc and copper*

According to the kinetic of mineral exchanges in organs, the zinc assessment was done every sample days in plasma and liver, every two days in muscle and every five days in femur. Hepatic, muscular, femoral and plasmatic zinc concentrations were measured by flame atomic absorption spectrometry (FAAS) with a Perkin-Elmer 560 model (Norwalk, CT, USA) with previously described methods [27]. Plasma was five fold diluted in hydrochloric acid 0.1M, whereas organs were wet ashed with nitric acid 14M. The ready to use 1 $g.L^{-1}$ Zinc stock standard solution (Merck, Darmstadt, Germany) was used to prepare calibrants in the range of 1 to 6 $\mu mol.L^{-1}$.

Plasma copper concentration was measured by FAAS with previously described methods [28]. Plasma was five fold diluted in 6 % n-butanol. A copper stock solution (Merck, Darmstadt, Germany) was used to prepare calibrants in the range of 1 to 6 $\mu mol.L^{-1}$. Hepatic, muscular and femoral copper concentrations were measured by electrothermal atomic absorption spectrometry (EAAS) after previous wet ashing with nitric acid 14M. A copper stock solution was used to prepare calibrants in the range of 0.4 to 1.6 $\mu mol.L^{-1}$ in nitric acid 0.1M.

Seronorm® trace element serum (Sero®, Billingstad, Norway) and NIST-SRM 1577b bovine liver powder (National Institute of Standards & Technology, Gaithersburg, MD) were used as internal quality control respectively for plasma and organs. In the organs, data were expressed as micrograms of Zn or Cu per gram of organ wet weight.

Statistical analysis

All results are expressed as means ± SEM. The global course of each parameter was evaluated by Kruskall and Wallis rank test and then the comparisons between controls and burned groups or between normal diet and depleted diet groups were performed with non-parametric rank sum tests (Mann and Whitney tests) using Statistica 7.1 software (StatSoft, France). Differences were considered statistically significant when p values were less than 0.05.

Results

Clinical observations

As shown in figure 1B, growth curves were not significantly different between the two groups of animals, even if the weight of the animals appeared slightly lower in zinc depleted group (ZD) than in control group (C). After six weeks of depleted zinc diet, ZD rats revealed a moderate depilation in regional skin areas without any other cutaneous symptom, contrary to clinical observations made after severe zinc deficiency (figure 1A).

Biological parameters before burn injury (table 2)

- *Trace elements status.* The 8 weeks diet led to a significant lower zinc level ($p < 0.01$) in femoral bone ($79 \pm 1.5\ \mu g.g^{-1}$) compared to control group ($113.3 \pm 4.6\ \mu g.g^{-1}$), whereas

differences between the two groups concerning plasma, liver and muscle zinc concentrations was effective but not statistically significant. For copper concentrations, no difference was observed after 8 weeks between the two groups in plasma, liver, muscle and femoral bone.

- *Oxidative stress parameters.* For TBARs, the zinc restriction induced in ZD rats a significant increase both in the plasma and the liver. Surprisingly, kidney TBARs were lower in ZD rats than in control animals. Muscle TBARs levels did not change whatever the diet was. GSH/GSSG was significantly lower in ZD rats (55.5 ± 9.9) compared to control rats (131 ± 29.8). Plasma, liver and kidney GPx and SOD levels as well as plasma total thiol groups were not altered by the zinc depletion before burn injury.

Evolution of biological parameters after burn injury

- *Zinc status (figure 2).*

After burn injury, plasma zinc level decreased in the two groups as soon as D1. Plasma zinc level never returned to initial value in control group (values significantly different from T0 until ten days after injury). The plasma zinc level decrease was enhanced in ZD rats within the 3 days following the burn injury. Possibly due to the relative low zinc level measured at T0 for the ZD group, the decrease was statically significant only within the three first days.

In liver, zinc level was not significantly altered by the burn injury within the first three days but increased significantly at D10 for control animals and more precociously from D5 to D10 for ZD rats. Ten days after burn, control rats presented a significant decrease of zinc level in femoral bone (99.3 ± 4.9 $\mu g.g^{-1}$) compared to T0 (115.9 ± 12.4 $\mu g.g^{-1}$). Compared to controls, bone zinc level was always lowered in ZD rats, all along the experiment ($p < 0.01$).

- *Oxidative stress parameters*

Plasma total thiol groups showed a significant decrease only in ZD group at D1, D8 and D10 (figure 3).

In the control group, we observed during the first 5 days of the experiment a statistically decrease of GSH/GSSG level compared to T0 (figure 4). In ZD group, GSH/GSSG level is also statistically lowered on D4 and D8 compared to T0. In addition, GSH/GSSG decrease was more important for ZD rats than for control rats as soon as D4 and GSH/GSSG tended to increase in controls from D5 to D10.

TBARs plasma level kinetic evolution was similar between normal and depleted series, first in plasma with a progressive decrease of TBARs values, and secondly in muscle with a significant decrease only at D5 (table 3). In liver, control group values were significantly higher than at T0, while the TBARs pattern was relatively stable in ZD group. In opposite, kidney TBARs pattern is unchanged in controls after burn injury, whereas it tended to increase and return to normal values until the end of the experiment for depleted rats.

Kidney and RBC GPx activity (figure 5) as well as RBC, liver and kidney SOD activity (data not shown) were not strongly disrupted by burn in depleted or control animals. In liver, the GPx activity tended to decrease within 3 days after burn, and zinc deficiency enhanced this decrease from D4 to D10.

Discussion

Burn injury is associated with enhanced systemic inflammatory reactions [29, 30] and oxygen radicals production [31, 32]. After burn injury, and more generally after trauma, imbalance between oxygen species production and free radicals scavengers determine the outcome of local and distant tissue damage, and further organ failure [33]. Among antioxidants trace elements, zinc acts in immune system [2], antioxidant mechanisms [34] and tissue repair [35]. A zinc deficient status will have deleterious effect on wound healing and on general recovery

of burned patients. In burned patients, zinc intakes could be essential in early protection against oxidative damages [13].

The animal model of thermal injury presents interesting advantages as choose age, diet and burn severity. It permits also blood and tissue sampling and a kinetic approach of the burn injury pathophysiology. Role of zinc status on metabolism was often examined with severe Zn deficiency (typically 0-2 ppm) induced in animals. The literature is full of short-term feeding studies [36-39].

Given that population does not suffer of a severe depletion, we decided, in this study, to induce a mild zinc deficiency (10 ppm) in order to mimic burned patients zinc status at their hospitalisation in Intensive Care Unit (ICU) and to avoid characteristic problems of severe zinc deficiency (weight loss, skin lesions, diarrhoea, healing impairments, genes expressions and immune dysfunctions) [40, 41]. After a diet of eleven weeks, we indeed did not observed, in depleted rats, major problems: weight loss was controlled, depilation was very moderate and absence of inflammation and copper metabolism perturbation due to Zinc/Copper interaction was checked by stable levels of this trace element. Consequently, we could assume that the modifications observed for oxidative stress parameters and for Zn status before burn injury were due to Zn different levels of the diet and not to inflammation status, growth impairment or differences in caloric intake.

In the present study, Zn subdeficient diet was moderately depleted in zinc, but enough to induce a zinc decrease in bone, one of the most important zinc exchangeable pools in the organism [42]. Previous studies have shown that zinc deficiency has detrimental effects on many bone biomechanical indices [43-45]. We observed that plasmatic and hepatic zinc homeostasis was ensured by the transfer from bone to these tissues conducing to low differences in liver and plasma and demonstrating the importance of a pool available to supply deficiencies in essential tissues or fluids. Low hepatic zinc would impair Cu/Zn

superoxide-dismutase function, thus allowing free radicals to damage hepatocyte function and structure, leading to hepatocyte necrosis and fibrosis. The existence of two pools of zinc in bone has been suggested by histochemical detection of free/loosely bound zinc ions in osteoid and osteoblasts, traced in matrix vesicles located in the undecalcified bone and in osteoblast [46]. As this pool is limited, with a diet moderately depleted in Zn, it could be postulate that, the organism only compensate to bearable limits.

Several studies demonstrated increased free radical production or increased oxidative damage in response to Zn deficiency *in vitro* or *in vivo* [47-49]. The present study also led to an increase of oxidative stress in ZD rats. In plasma and liver higher levels of endogenous TBARs were found, suggesting an increased lipid peroxidation in ZD rats. In parallel, GSH/GSSG ratio was decreased indicating that reduced glutathione pool could be altered by oxidative damage. Moreover, vulnerability to free radical damage in a number of cell systems has been reported in case of GSH depletion [50]. The glutathione redox couple is an actor of free radicals scavenging but, plays also an important role both in the rate of zinc transfer and in the ultimate number of zinc atoms transferred from metallothioneins [51]. Under physiological GSH concentrations, Zn transfer from metallothioneins is totally inhibited and GSSG induces a 3-fold increase of the rate of zinc transfer, leading to a total release of the Zn atoms [52]. Hence, GSSG concentration is a major determinant for efficient Zn transfer. Finally, GSH/GSSG ratio modulates Zn transfer through the redox state. In summary, this system allows a subtle regulation of intracellular Zn movements able to balance nutritional intakes and physiological needs.

Impact of low zinc status on burn-induced oxidative stress

The observed decrease in plasma Zn in control group after burn injury was already reported by others [10, 11]. Multiple possible mechanisms could be involved in the Zn status response

after thermal injury. A loss of Zn from the skin, where 20 % of the body's zinc is stored, has been demonstrated [53]. Zn modification can also be related to the generalization of the burned-induced inflammatory response [54, 55]. The immune cells (macrophages, polymorphonuclear cells and lymphocytes) secrete inflammatory mediators including cytokines and molecules such as nitric oxide and platelet-activating factor, which extend the inflammatory response to the whole system. A large increase of serum IL-6 was observed in burn patients without infections [56]. It was also observed an increase of IL1 during the acute phase response in correlation with a marked depression in the serum Zn [57]. IL-1 and IL-6 stimulate the hepatic acute-phase response proteins implicated in homeostasis preservation [58, 59] and stimulate the production of metallothioneins by JAK/STAT transduction pathway [60]. Metallothioneins are induced and implicated in transport of Zn from blood to the hepatic compartments [61] and, consequently, an enhancement of hepatic Zn participates to the defence against oxidative damage. Our results are in agreement with these data: one day after burn injury, serum Zn reached a nadir and never returned to control values whereas liver Zn level progressively increased.

The interesting point is to compare the evolution of liver Zn level in the two groups. In control group, Zn level shows a constant increase from day 3 to 10. In contrast, in ZD rats we observed a progressive restoration of liver Zn level within the five first days which remained unchanged compared to the initial value of control group. In the same time, the plasma Zn level was not statistically different between the two groups. Our results (data not shown) indicate that other organs are not involved in zinc mobilization. Consequently, it can be postulated that the only cause for liver Zn could be the bone pool. Indeed, the animals of the control group could have used their free bone pool in aid of critical tissues. In contrast, ZD rats could not have the possibility to increase their liver Zn level over the baseline because this pool is probably at the lowest limit for these animals.

In the present study, decrease of GSH/GSSG ratio, after thermal injury, in both normal and depleted rats could induce an enhancement of Zn transfer towards liver. We can notice that depleted rats have, in most case, a lower ratio and so, a possible more important Zn transfer rate. This fact is suitable with the larger decreases observed in Zn plasma and bone levels in depleted rats.

Physiological properties of glutathione could explain the change of GSH/GSSG ratio. As an intrinsic antioxidant, GSH protect against oxidative stress by spontaneous reactions with free radicals. It also serves as a substrate of GPx and plays an important role in the maintenance of protein sulfhydryl groups [62]. In our study, the decrease of GSH, higher in depleted rats, might suggest that a Zn deficiency worsen antioxidant status after a burn.

After burn injury, within the first week, neither GPx nor SOD activity were modified by zinc deficiency. In liver, the observed decline in GPx activity might be due to modification of the sulfhydryl groups in this enzyme by oxygen free radicals or by TBARs [63]. The impaired oxidative status of depleted rats would lead to a liver GPx malfunctioning. In kidney, we previously observed the relative stability of GPX [11]. GPx synthesis taking place in kidney, the trauma certainly induced an increased GPx production in order to counteract free radicals. Moreover, after burn, a Se mobilization was noted in the kidney [64], Se being used to maintain GPx synthesis. In liver, controversial results were found about GPX activity after burn injury. In this study, Zn deficiency leads to a lower liver GPx activity (D5 and D10). In agreement with us, Sabeh et al. noted a GPx decrease in liver in a rabbit model of burn when, in a mice model, Kaway et al. observed that GPx activity increase in the liver only from 4 to 24 hours after burn injury [65, 66]. In previous studies, we demonstrated an increase of SOD in reaction to the decrease of GPx in selenium deficient rats [67]. Here, probably related to the stability of GPx, no modification in SOD activity was observed. Moreover, because in Cu/Zn

SOD the zinc atoms have only a structural function, moderate zinc deficiency could not impair Cu/Zn SOD activity.

Plasma TBARs are increased in ZD rats compared to control group before burn. This data confirms the deleterious effect of zinc deficiency in increasing lipid peroxidation, as already reported in animals [68-70]. The evolution of plasma TBARs after burn showed an unexpected decrease in the two groups. However, Lipid peroxidation after burn injury has been largely documented in humans [31, 71, 72] and animals [73, 74]. In the present study and contrastly to selenium [67], zinc status does not seem to be determinant to protect lipids from burn-induced free radical production.

After burn injury, the SH groups decrease in ZD animals suggesting an enhanced protein oxidation**.** The protection of plasma protein sulhydryl groups is thought to involve reduction of sulfhydryl reactivity through one of three mechanisms: (1) direct binding of Zn to the sulfhydryl; (2) steric hindrance as a result of binding to some other protein site in close proximity to the sulfhydryl group; (3) a conformational change from binding to some other site on the protein. Hence, a Zn deficiency can easily lead to an impaired protection of thiol groups.

In conclusion, our results, observed in burned rats with moderate depleted zinc diet, show that even if zinc intakes modulate the response to burn injury regarding glutathione and SH groups, a depleted status is not a major component to be considered as aggravating factor toward the burn induced damages. These preliminary data needs to go deeply into. However, given the beneficial effects of an optimal zinc status reported in burned patients or animal models, the positive role of zinc supplementation demonstrated in clinical recovery should be enlightened.

Acknowledgements

We thank H.Chaussard, D.Coulon, A.Zocaro and J.Denis (CRSSA, Grenoble) for their excellent technical assistance.

This work was supported by a grant from Délégation Générale à l'Armement (DGA, France).

Conflict of interest statement

The authors have no financial or proprietary interest in the subject matter.

References

1. Powell SR (2000) The antioxidant properties of zinc. J Nutr 130(5S Suppl):1447S-54S
2. Hirano T, Murakami M, Fukada T, Nishida K, Yamasaki S, Suzuki T (2008) Roles of Zinc and Zinc Signaling in Immunity: Zinc as an Intracellular Signaling Molecule. Adv Immunol 97C:149-76
3. Dreosti IE (2001) Zinc and the gene. Mutat Res 475(1-2):161-7
4. Witkiewicz-Kucharczyk A, Bal W (2006) Damage of zinc fingers in DNA repair proteins, a novel molecular mechanism in carcinogenesis. Toxicol Lett 162(1):29-42
5. Yan M, Song Y, Wong CP, Hardin K, Ho E (2008) Zinc deficiency alters DNA damage response genes in normal human prostate epithelial cells. J Nutr 138(4):667-73
6. Marklund SL, Westman NG, Lundgren E, Roos G (1982) Copper- and zinc-containing superoxide dismutase, manganese-containing superoxide dismutase, catalase, and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res 42(5):1955-61
7. Sato M, Bremner I (1993) Oxygen free radicals and metallothionein. Free Radic Biol Med 14(3):325-37
8. Della Rovere F, Granata A, Pavia R, Tomaino A, Zirilli A, Monaco F, Familiari D, La Rocca A, Ientile R, Mondello B, Basile G, Monaco M (2004) Vitamins A, E, microelements and membrane lipid peroxidation in patients with neoplastic disease treated with calcium antagonists and antagonists of receptors H2. Anticancer Res 24(3a):1449-53
9. Zago MP, Oteiza PI (2001) The antioxidant properties of zinc: interactions with iron and antioxidants. Free Radic Biol Med 31(2):266-74
10. Selmanpakoglu AN, Cetin C, Sayal A, Isimer A (1994) Trace element (Al, Se, Zn, Cu) levels in serum, urine and tissues of burn patients. Burns 20(2):99-103
11. Agay D, Anderson RA, Sandre C, Bryden NA, Alonso A, Roussel AM, Chancerelle Y (2005) Alterations of antioxidant trace elements (Zn, Se, Cu) and related metallo-enzymes in plasma and tissues following burn injury in rats. Burns 31(3):366-71
12. Voruganti VS, Klein GL, Lu HX, Thomas S, Freeland-Graves JH, Herndon DN (2005) Impaired zinc and copper status in children with burn injuries: need to reassess nutritional requirements. Burns 31(6):711-6
13. Berger MM, Baines M, Raffoul W, Benathan M, Chiolero RL, Reeves C, Revelly JP, Cayeux MC, Senechaud I, Shenkin A (2007) Trace element supplementation after major

burns modulates antioxidant status and clinical course by way of increased tissue trace element concentrations. Am J Clin Nutr 85(5):1293-300

14. Berger MM, Shenkin A (1998) Trace elements in trauma and burns. Curr Opin Clin Nutr Metab Care 1(6):513-7
15. Prasad AS (1996) Zinc deficiency in women, infants and children. J Am Coll Nutr 15(2):113-20
16. King JC, Shames DM, Woodhouse LR (2000) Zinc homeostasis in humans. J Nutr 130(5S Suppl):1360S-6S
17. Pennington JA (1996) Intakes of minerals from diets and foods: is there a need for concern? J Nutr 126(9 Suppl):2304S-2308S
18. Walker HL, Mason AD, Jr. (1968) A standard animal burn. J Trauma 8(6):1049-51
19. Richard MJ, Portal B, Meo J, Coudray C, Hadjian A, Favier A (1992) Malondialdehyde kit evaluated for determining plasma and lipoprotein fractions that react with thiobarbituric acid. Clin Chem 38(5):704-9
20. Faure P, Lafond JL (1995) Measurement of plasma sulfhydryl and carbonyl groups as a possible indicator of protein oxidation. in Favier AE, Cadet J, Kalnyanaraman M, Fontecave M, Pierre JL (eds): Analysis of free radicals in biological systems. Birkäuser, Basel, Boston, Berlin, pp 237-248.
21. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150(1):76-85
22. Akerboom TP, Sies H (1981) Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. Methods Enzymol 77:373-82
23. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70(1):158-69
24. Woolliams JA, Wiener G, Anderson PH, McMurray CH (1983) Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Res Vet Sci 34(3):253-6
25. Arthur JR, Boyne R (1985) Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci 36(16):1569-75
26. International committee for standardization in haematology (1967) Recommendations for haemoglobinometry in human blood. Br J Haematol 13:71-5
27. Arnaud J, Bellanger J, Bienvenu F, Chappuis P, Favier A (1986) Recommended method for assaying serum zinc with flame atomic absorption. Ann Biol Clin 44(1):77-87

28. Arnaud J, Bellanger J, Chappuis P, Favier A, Galliot M (1985) Recommandations for the assay of serum copper by flame atomic absorption spectrometry. Ann Biol Clin 43:297-318
29. Sabeh F, Hockberger P, Sayeed MM (1998) Signaling mechanisms of elevated neutrophil O2- generation after burn injury. Am J Physiol 274(2 Pt 2):R476-85
30. Reyes R, Jr., Wu Y, Lai Q, Mrizek M, Berger J, Jimenez DF, Barone CM, Ding Y (2006) Early inflammatory response in rat brain after peripheral thermal injury. Neurosci Lett 407(1):11-5
31. Pintaudi AM, Tesoriere L, D'Arpa N, D'Amelio L, D'Arpa D, Bongiorno A, Masellis M, Livrea MA (2000) Oxidative stress after moderate to extensive burning in humans. Free Radic Res 33(2):139-46
32. Gurbuz V, Corak A, Yegen BC, Kurtel H, Alican I (1997) Oxidative organ damage in a rat model of thermal injury: the effect of cyclosporin A. Burns 23(1):37-42
33. Farriol M, Fuentes F, Venereo Y, Solano I, Orta X, Segovia T (2001) Antioxidant capacity in severely burned patients. Pathol Biol (Paris) 49(3):227-31
34. Jing MY, Sun JY, Zi NT, Sun W, Qian LC, Weng XY (2007) Effects of zinc on hepatic antioxidant systems and the mRNA expression levels assayed by cDNA microarrays in rats. Ann Nutr Metab 51(4):345-51
35. Casey G (2003) Nutritional support in wound healing. Nurs Stand 17(23):55-8
36. Eberle J, Schmidmayer S, Erben RG, Stangassinger M, Roth HP (1999) Skeletal effects of zinc deficiency in growing rats. J Trace Elem Med Biol 13(1-2):21-6
37. Hosea HJ, Taylor CG, Wood T, Mollard R, Weiler HA (2004) Zinc-deficient rats have more limited bone recovery during repletion than diet-restricted rats. Exp Biol Med (Maywood) 229(4):303-11
38. Bruno RS, Song Y, Leonard SW, Mustacich DJ, Taylor AW, Traber MG, Ho E (2007) Dietary zinc restriction in rats alters antioxidant status and increases plasma F2 isoprostanes. J Nutr Biochem 18(8):509-18
39. Oteiza PI, Olin KL, Fraga CG, Keen CL (1995) Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. J Nutr 125(4):823-9
40. Cuevas LE, Koyanagi A (2005) Zinc and infection: a review. Ann Trop Paediatr 25(3):149-60
41. Prasad AS (2008) Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exp Gerontol 43(5):370-7

42. Giugliano R, Millward DJ (1984) Growth and zinc homeostasis in the severely Zn-deficient rat. Br J Nutr 52(3):545-60
43. Salgueiro MJ, Torti H, Meseri E, Weill R, Orlandini J, Urriza R, Zubillaga M, Janjetic M, Barrado A, Boccio J (2006) Dietary zinc effects on zinc, calcium, and magnesium content in bones of growing rats. Biol Trace Elem Res 110(1):73-8
44. Peretz A, Papadopoulos T, Willems D, Hotimsky A, Michiels N, Siderova V, Bergmann P, Neve J (2001) Zinc supplementation increases bone alkaline phosphatase in healthy men. J Trace Elem Med Biol 15(2-3):175-8
45. Yamaguchi M, Ehara Y (1995) Zinc decrease and bone metabolism in the femoral-metaphyseal tissues of rats with skeletal unloading. Calcif Tissue Int 57(3):218-23
46. Danscher G, Mosekilde L, Rungby J (1999) Histochemical detection of zinc in mineralizing rat bone: autometallographic tracing of zinc ions in the mineralization front, osteocytes and asteoblasts. J Histotechnol 22:85-90
47. Oteiza PI, Clegg MS, Zago MP, Keen CL (2000) Zinc deficiency induces oxidative stress and AP-1 activation in 3T3 cells. Free Radic Biol Med 28(7):1091-9
48. Ho E, Ames BN (2002) Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. Proc Natl Acad Sci U S A 99(26):16770-5
49. Bray TM, Kubow S, Bettger WJ (1986) Effect of dietary zinc on endogenous free radical production in rat lung microsomes. J Nutr 116(6):1054-60
50. Reed DJ (1990) Glutathione: toxicological implications. Annu Rev Pharmacol Toxicol 30:603-31
51. Jiang LJ, Maret W, Vallee BL (1998) The glutathione redox couple modulates zinc transfer from metallothionein to zinc-depleted sorbitol dehydrogenase. Proc Natl Acad Sci U S A 95(7):3483-8
52. Maret W (1994) Oxidative metal release from metallothionein via zinc-thiol/disulfide interchange. Proc Natl Acad Sci U S A 91(1):237-41
53. Berger MM, Cavadini C, Bart A, Mansourian R, Guinchard S, Bartholdi I, Vandervale A, Krupp S, Chiolero R, Freeman J, et al. (1992) Cutaneous copper and zinc losses in burns. Burns 18(5):373-80
54. Bloemsma GC, Dokter J, Boxma H, Oen IM (2008) Mortality and causes of death in a burn centre. Burns
55. Faunce DE, Llanas JN, Patel PJ, Gregory MS, Duffner LA, Kovacs EJ (1999) Neutrophil chemokine production in the skin following scald injury. Burns 25(5):403-10

56. Yeh FL, Lin WL, Shen HD, Fang RH (1999) Changes in circulating levels of interleukin 6 in burned patients. Burns 25(2):131-6
57. Darlington GJ, Wilson DR, Lachman LB (1986) Monocyte-conditioned medium, interleukin-1, and tumor necrosis factor stimulate the acute phase response in human hepatoma cells in vitro. J Cell Biol 103(3):787-93
58. Le JM, Vilcek J (1989) Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response. Lab Invest 61(6):588-602
59. Castell JV, Gomez-Lechon MJ, David M, Andus T, Geiger T, Trullenque R, Fabra R, Heinrich PC (1989) Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 242(2):237-9
60. Lee DK, Carrasco J, Hidalgo J, Andrews GK (1999) Identification of a signal transducer and activator of transcription (STAT) binding site in the mouse metallothionein-I promoter involved in interleukin-6-induced gene expression. Biochem J 337(Pt 1)):59-65
61. Beattie H, Bremner I (2000) Metallothioneins: their cellular function and relationship with zinc. in Roussel AM, Anderson RA, Favier AE (eds): Trace elements in man and animals. New York, Kluwer Academic/ Plenum Publishers, pp 961-967.
62. Meister A, Anderson ME (1983) Glutathione. Annu Rev Biochem 52:711-60
63. Pigeolet E, Remacle J (1991) Susceptibility of glutathione peroxidase to proteolysis after oxidative alteration by peroxides and hydroxyl radicals. Free Radic Biol Med 11(2):191-5
64. Sandre C, Agay D, Ducros V, Van Uye A, Cruz C, Chancerelle Y, Roussel AM (2004) Early evolution of selenium status and oxidative stress parameters in rat models of thermal injury. J Trace Elem Med Biol 17(4):313-8
65. Sabeh F, Baxter CR, Norton SJ (1995) Skin burn injury and oxidative stress in liver and lung tissues of rabbit models. Eur J Clin Chem Clin Biochem 33(6):323-8
66. Kawai S, Komura J, Asada Y, Niwa Y (1988) Experimental burn-induced changes in lipid peroxide levels, and activity of superoxide dismutase and glutathione peroxidase in skin lesions, serum, and liver of mice. Arch Dermatol Res 280(3):171-5
67. Sandre C, Agay D, Ducros V, Faure H, Cruz C, Alonso A, Chancerelle Y, Roussel AM (2006) Kinetic changes of oxidative stress and selenium status in plasma and tissues following burn injury in selenium-deficient and selenium-supplemented rats. J Trauma 60(3):627-34
68. Hidalgo MC, Exposito A, Palma JM, de la Higuera M (2002) Oxidative stress generated by dietary Zn-deficiency: studies in rainbow trout (Oncorhynchus mykiss). Int J Biochem Cell Biol 34(2):183-93

69. Miceli MV, Tate DJ, Jr., Alcock NW, Newsome DA (1999) Zinc deficiency and oxidative stress in the retina of pigmented rats. Invest Ophthalmol Vis Sci 40(6):1238-44
70. Shaheen AA, el-Fattah AA (1995) Effect of dietary zinc on lipid peroxidation, glutathione, protein thiols levels and superoxide dismutase activity in rat tissues. Int J Biochem Cell Biol 27(1):89-95
71. Kumar R, Seth RK, Sekhon MS, Bhargava JS (1995) Serum lipid peroxide and other enzyme levels of patients suffering from thermal injury. Burns 21(2):96-7
72. Ritter C, Andrades M, Guerreiro M, Zavaschi L, Gelain DP, Souza LF, Ribeiro CA, Clausell N, Menna-Barreto S, Moreira JC, Dal-Pizzol F (2003) Plasma oxidative parameters and mortality in patients with severe burn injury. Intensive Care Med 29(8):1380-3
73. Sener G, Satyroglu H, Ozer Sehirli A, Kacmaz A (2003) Protective effect of aqueous garlic extract against oxidative organ damage in a rat model of thermal injury. Life Sci 73(1):81-91
74. Altavilla D, Galeano M, Bitto A, Minutoli L, Squadrito G, Seminara P, Venuti FS, Torre V, Calo M, Colonna M, Lo Cascio P, Giugliano G, Scuderi N, Mioni C, Leone S, Squadrito F (2005) Lipid peroxidation inhibition by raxofelast improves angiogenesis and wound healing in experimental burn wounds. Shock 24(1):85-91

Legends for illustrations

Table 1. Ingredients for the two diets.

Table 2. Oxidative and trace element status of zinc depleted rats (ZD) compared to control rats (C) before burn injury.

Table 3. Evolution of TBARs levels in plasma and organs of Zn depleted (ZD) and Zn control (C) rats after burn injury.

Figure 1. Experimental model of zinc subdeficiency.

Figure 2. Evolution of plasma, liver and femoral zinc levels in both control rats (C) and zinc depleted rats (ZD) after burn injury.

Figure 3. Evolution of thiol groups in plasma of both control rats (C) and zinc rats (ZD) after burn injury.

Figure 4. Evolution of GSH / GSSG ratio in both control (C) and zinc depleted (ZD) rats after burn injury.

Figure 5. Evolution of RBC, liver and kidney glutathione peroxidase levels after burn injury in control (C) and zinc depleted (ZD) rats.

Table 1. Ingredients for the two diets.

Dextrose	59 %
Egg white	20 %
Cellulose	6 %
Corn oil	5 %
Colza oil	2 %
Zinc gluconate	80 $mg.kg^{-1}$ for Zn adequate diet 10 $mg.kg^{-1}$ for Zn depleted diet
Vitamin and mineral premix	
The mineral premix was prepared without any zinc	

Table 2. Oxidative and trace element status of zinc depleted rats (ZD) compared to control rats (C) before burn injury.

			Initial values (T0)	
			Control (C)	Zinc depleted (ZD)
Zinc	Plasma	($\mu g.mL^{-1}$)	1.07 ± 0.11	0.89 ± 0.18
	Liver	($\mu g.g^{-1}$ wet weight)	35.9 ± 8.0	27.4 ± 3.1
	Muscle	($\mu g.g^{-1}$ wet weight)	7.3 ± 1.0	6.7 ± 1.1
	Bone	($\mu g.g^{-1}$ wet weight)	115.9 ± 12.5	82.1 ± 5.6 ##
Copper	Plasma	($\mu g.mL^{-1}$)	1.16 ± 0.15	1.01 ± 0.08
	Liver	($\mu g.g^{-1}$ wet weight)	5.6 ± 1.6	5.3 ± 0.5
	Muscle	($\mu g.g^{-1}$ wet weight)	1.06 ± 0.34	1.00 ± 0.16
	Bone	($\mu g.g^{-1}$ wet weight)	0.77 ± 0.04	0.75 ± 0.08
Plasma thiols groups		($\mu mol.g^{-1}$ proteins)	5.58 ± 1.25	5.43 ± 0.34
Reduced glutathione / oxidized glutathione ratio			131.6 ± 66.7	55.5 ± 24.3 #
Thiobarbituric acid reactive species (TBARs)	Plasma	(μmole)	3.86 ± 0.5	5.54 ± 1.11 #
	Liver	($\mu mol.g^{-1}$ proteins)	0.16 ± 0.03	0.23 ± 0.05 #
	Kidney	($\mu mol.g^{-1}$ proteins)	0.21 ± 0.04	0.13 ± 0.04 ##
	Muscle	($\mu mol.g^{-1}$ proteins)	0.30 ± 0.05	0.37 ± 0.1
superoxide dismutase (SOD)	Red blood cells ($U.g^{-1}$ Hb)		2497 ± 144	2465 ± 55
	Liver	($U.g^{-1}$ proteins)	18988 ± 1850	21144 ± 1703
	Kidney	($U.g^{-1}$ proteins)	13.35 ± 0.93	12.17 ± 0.28 #
Glutathione peroxidase (GPx)	Red blood cells ($U.g^{-1}$ Hb)		564 ± 61.2	563.2 ± 89.8
	Liver	($U.g^{-1}$ proteins)	1558.7 ± 152.7	1544 ± 117.7
	Kidney	($U.g^{-1}$ proteins)	1169.2 ± 104.6	1107.3 ± 134.4

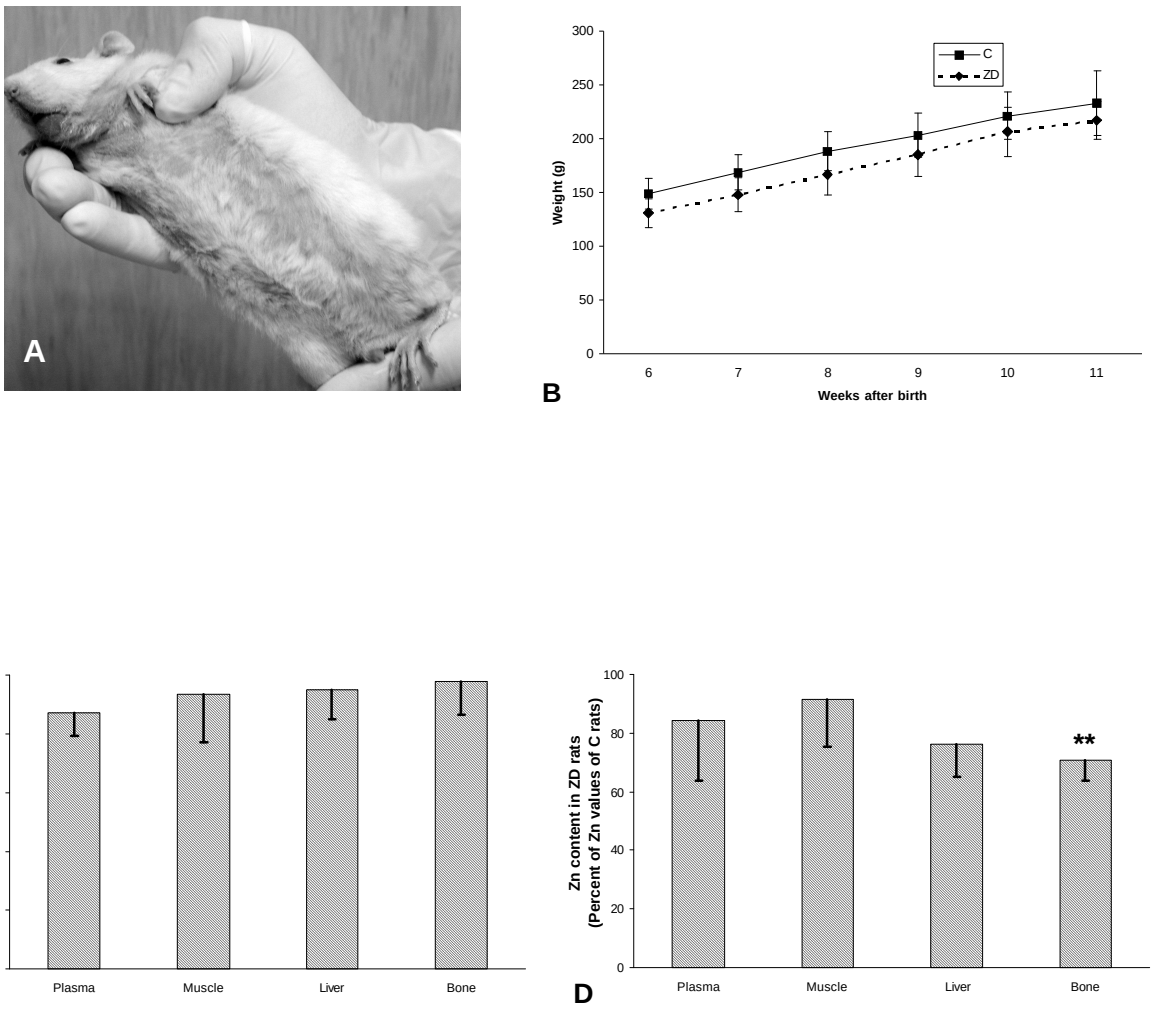
Data are presented as means ± SD (n=5).
# $p < 0.05$ or ## $p < 0.01$ compared to the control group.

Table 3. Evolution of TBARs levels in plasma and organs of Zn depleted (ZD) and Zn control (C) rats after burn injury.

		T0	D1	D3	D5	D8	D10
Plasma	ZD	5.54 ± 1.11	3.83 ± 1.87	3.02 ± 0.19**	3.25 ± 0.72**	4.23 ± 1.14	3.63 ± 0.44**
	C	3.86 ± 0.5 #	3.87 ± 0.79	3.38 ± 0.22	2.88 ± 0.47*	2.99 ± 0.17**	3.16 ± 0.26*
Liver	ZD	0.23 ± 0.05	0.27 ± 0.04	0.26 ± 0.04	0.26 ± 0.06	0.24 ± 0.04	0.24 ± 0.04
	C	0.16 ± 0.03 #	0.25 ± 0.05*	0.22 ± 0.03*	0.25 ± 0.05*	0.27 ± 0.05**	0.24 ± 0.06*
Kidney	ZD	0.13 ± 0.04	0.20 ± 0.03**	0.22 ± 0.03**	0.26 ± 0.03**	0.22 ± 0.02**	0.22 ± 0.04*
	C	0.21 ± 0.04 ##	0.21 ± 0.05	0.22 ± 0.03	0.24 ± 0.02	0.23 ± 0.04	0.22 ± 0.03
Muscle	ZD	0.37 ± 0.1	0.46 ±0.14	0.47 ± 0.03	0.25 ± 0.04*	0.34 ± 0.07	0.32 ± 0.07
	C	0.30 ± 0.05	0.38 ± 0.02*	0.32 ± 0.03	0.19 ± 0.03**	0.30 ± 0.08	0.27 ± 0.06

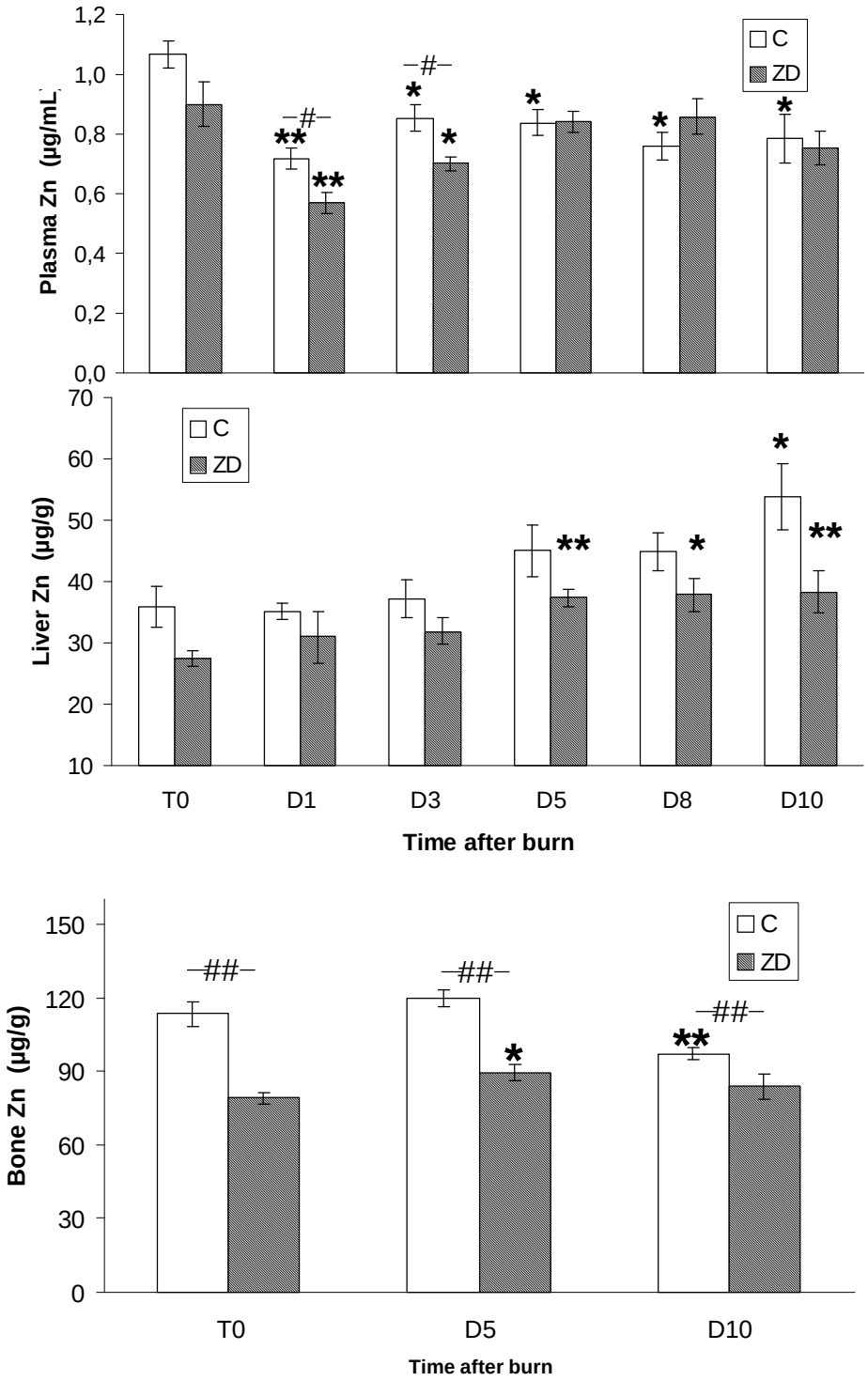
Data are presented as means (n = 5) ± SD and are expressed as the ratio of organ TBARs mean values *vs.* organs proteins mean values for each group of rats (µmol.L^{-1}.g^{-1} proteins) or as plasma TBARs mean values for each group of rats (µmol.L^{-1}).
* $p < 0.05$ or ** $p < 0.01$ compared to initial values (T0).
# $p < 0.05$ or ## $p < 0.01$ control series versus Zn depleted series at T0.

Figure 1. Experimental model of zinc subdeficiency.



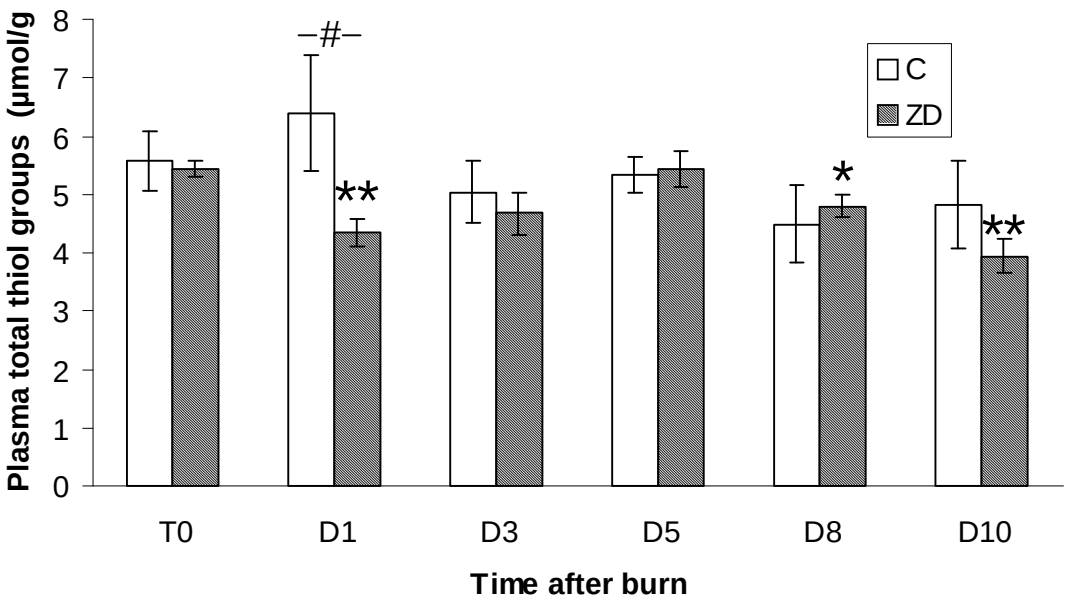
(A): Absence of major clinical side effects.
(B): Growth curve of control [C] and zinc depleted [ZD] rats before injury. Data are presented as means ± SD (n = 40).
(C) and (D): Copper and zinc assessed in plasma and organs of zinc depleted animals [ZD]. Trace element levels are presented as mean ratio ± SD (n=5) of ZD values to control rats values. **: $p < 0.01$ compared to the control values (100 %).

Figure 2. Evolution of plasma, liver and femoral zinc levels in both control rats (C) and zinc depleted rats (ZD) after burn injury.



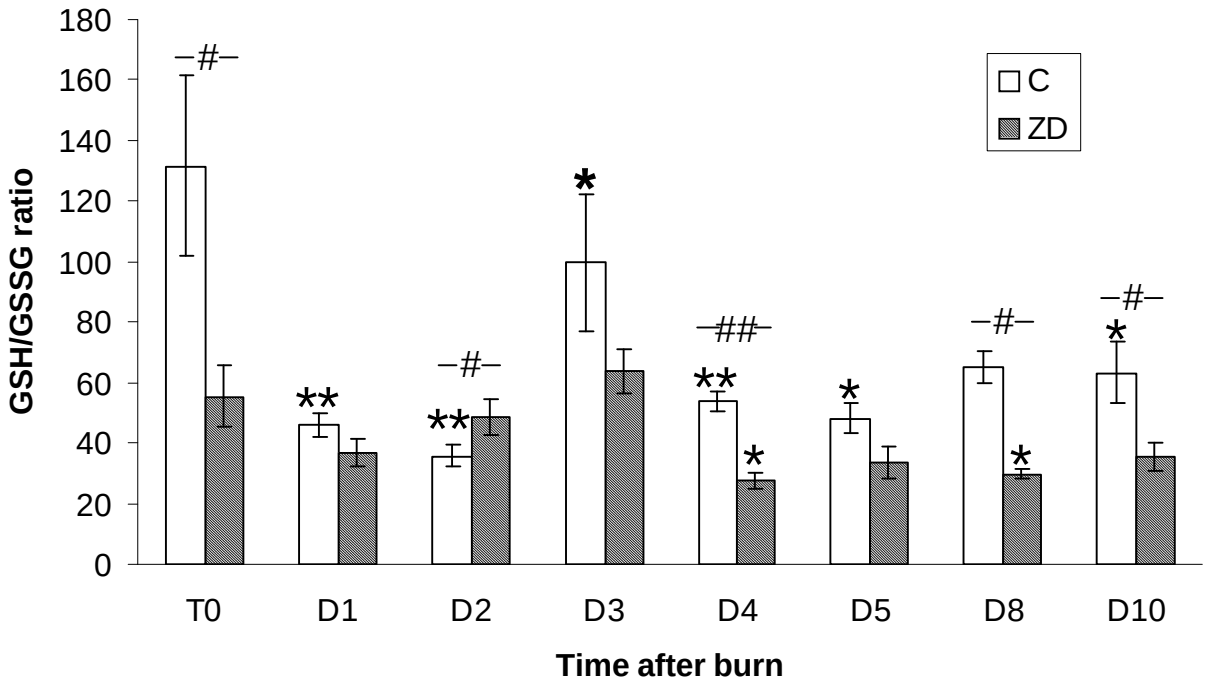
Data are presented as means ± SEM (n=5) and are expressed, in organs, on a wet weight basis.
* $p < 0.05$ or ** $p < 0.01$: comparison to the initial value (T0).
# $p < 0.05$ or ## $p < 0.01$: comparison between (C) and (ZD) series.

Figure 3. Evolution of thiol groups in plasma of both control rats (C) and zinc depleted rats (ZD) after burn injury.



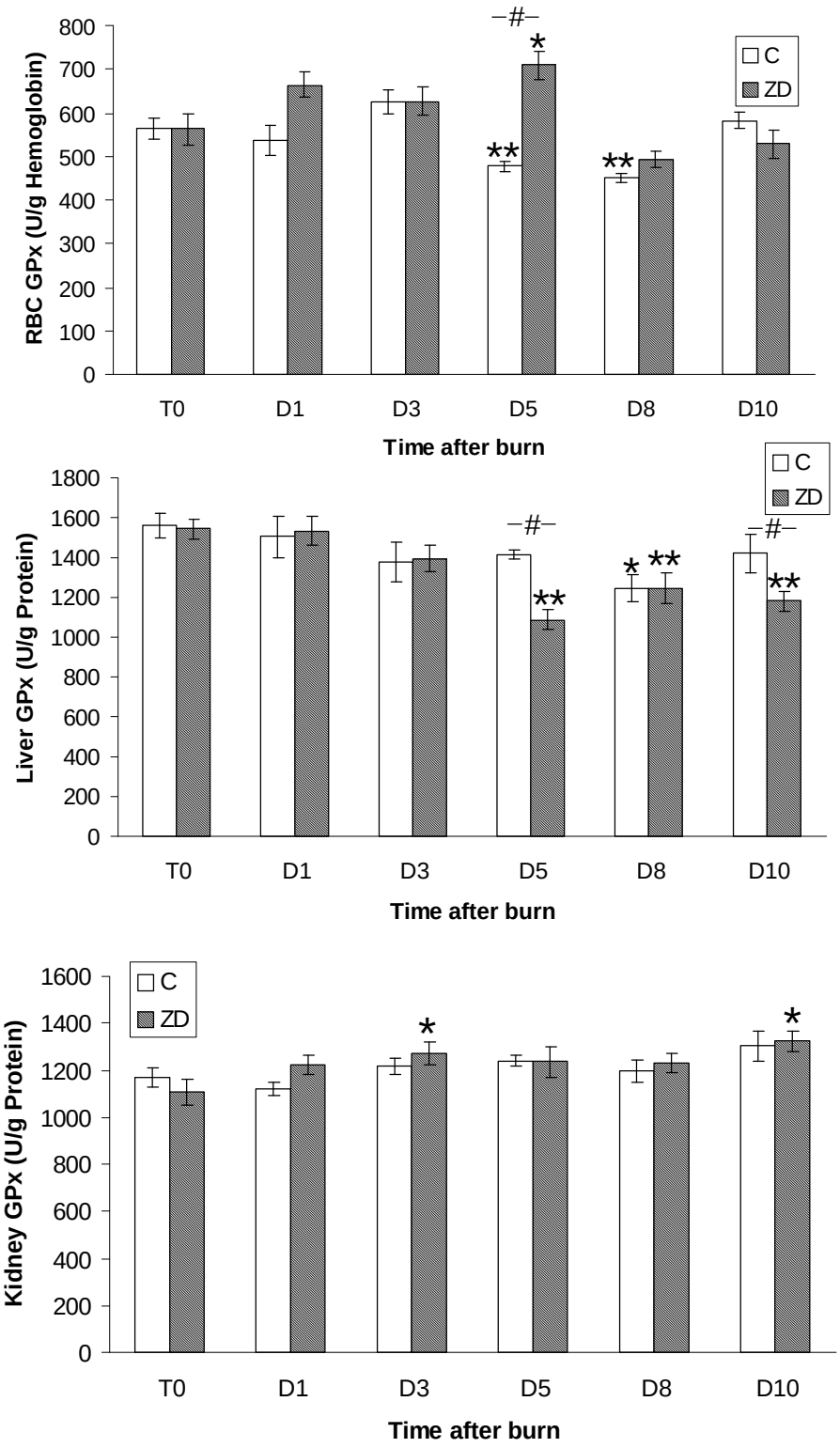
Data are presented as means ± SEM (n=5).
* $p < 0.05$ or ** $p < 0.01$: comparison to the initial value (T0).
# $p < 0.05$: comparison between (C) and (ZD) series.

Figure 4. Evolution of GSH / GSSG ratio in both control (C) and zinc depleted (ZD) rats after burn injury.



Data are presented as means ± SEM (n=5).
* $p < 0.05$ or ** $p < 0.01$: comparison to the initial value (T0).
# $p < 0.05$ or ## $p < 0.01$: comparison between (C) and (ZD) series.

Figure 5. Evolution of RBC, liver and kidney glutathione peroxidase levels after burn injury in control (C) and zinc depleted (ZD) rats.



Data are presented as means ± SEM (n=5).
* $p < 0.05$ or ** $p < 0.01$: comparison to the initial value (T0).
# $p < 0.05$: comparison between (C) and (ZD) series.