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The authors declare to have no personal, financial or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence, or be perceived to influence, the work submitted.

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Abbreviations : AFR, ascorbyl free radical; AL, alpha-lipoic acid; ANOVA, analysis of variance; AU, arbitrary units; DHE, dihydroethidium; DHLA, dihydrolipoic acid; DOX , Doxorubicin; ESR, electron spin resonance; HR, heart rate; Ht, hematocrit; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; $O_2^{\bullet-}$, superoxide anion; ROOH, hydroperoxides; TBARs, thiobarbituric acid reactive substances

1. Introduction

Doxorubicin is an anthracycline anti-neoplasic drug used in the treatment of a wide range of solid tumors and of leukemia in children and adults. Despite its good therapeutic results, the clinical use of doxorubicin during chemotherapy is limited since it induces acute or sub-acute, early chronic or late chronic cardiotoxicity [1-2]. Cardiac alterations become more obvious several years after the end of the treatment and may occur as dilated cardiomyopathy leading to congestive heart failure [3-6].

Despite a large amount of research on this subject, the molecular mechanisms involved in chronic anthracycline cardiotoxicity remain a major topic of discussion. Several aspects of this phenomenon, such as apoptosis or the alteration of iron and calcium homeostasis have been described but the exact mechanism is not yet fully understood. Indeed, oxidative stress is believed to be an important pathway involved in the cardiac side-effects of anthracycline therapy [7-9]. It is widely known that superoxide anion free radical ($O_2^{\bullet -}$) is generated during the "redox cycling" of anthracycline or after oxido-reduction processes taking place inside the anthracycline-iron complex. Then, $O_2^{\bullet -}$ is converted to hydroxyl radical ($HO^{\bullet}$) in the presence of transition metals or forms peroxinitrite ($ONOO^-$) in the presence of nitric oxide ($^{\bullet}NO$). Both of these are strong oxidants which can induce cellular injury [9-11].

Along with preventive measures implemented to limit anthracycline-induced cardiotoxicity [4, 12], several antioxidant molecules have been tested for their potential cardioprotective effects in different experimental models in the last forty years. Despite some beneficial results of vitamin E, vitamin A, N-acetyl cysteine, probucol [13], carvedilol, glutathione, superoxide dismutase, catalase, none of these antioxidants has proved to be clearly beneficial in humans [14-15]. The only drug presently approved for use in clinical practice to reduce the cardiac side-effects of anthracyclines is dexrazoxane (Cardioxane®) [15-17], a prodrug of ADR-925, a compound with a chemical structure close to that of ethylenediamine tetra-acetic acid, which chelates intracellular iron [15, 18-19].

In this context, it seems interesting to evaluate the possible cardioprotective effects of alpha-lipoic acid (AL). Alpha-lipoic acid or thioctic acid (6,8-dithio-octanoic) is a thiol compound with antioxidant properties which can be found in plants and animals. AL is able to scavenge free radicals ($HO^{\bullet}$, HClO, $^1O_2$), chelate transition metals (iron and copper) or regenerate oxidized forms of antioxidants (vitamin E, vitamin C and glutathione) [20-21]. AL is both water-

and lipid-soluble, a property which allows it to concentrate in cellular and extracellular environments. Exogenous AL is rapidly absorbed from the diet, and is reduced inside the cell to dihydrolipoic acid (DHLA), the most active form of the substance [22-23]. Its low negative redox potential (0.32 V) gives AL/DLHA strong reducing properties. A number of studies have reported the beneficial effects of AL in *diabetes mellitus*, insulin resistance [24], diabetic neuropathy [25-26], vascular inflammation [27-28], erythrocyte membrane stability [29] and neurodegenerative diseases [23, 30]. All these effects are associated with its antioxidant properties. However, a few recent studies have reported that the pro-oxidant potential of AL and DHLA depends on the type of oxidative stress and the physiological conditions [31-32].

In this general context, the aims of our work were: 1) to create a model of chronic doxorubicin-induced cardiotoxicity in rats, 2) to confirm the physiopathological role of oxidative stress in cardiac injury and 3) to assess the impact of treatment with alpha-lipoic acid (AL) in these experimental conditions.

2. Materials and methods

2.1. Animals and experimental protocol

The local ethics committee approved the experimental protocol and the investigators complied with authorization 6007 from the French government, which agrees with EC Directive 86/609: EEC for animal experiments. For the purpose of our study, 72 male Wistar rats (Charles-River, L'Abresle, France; 300-350 g at the beginning of the experiment) were divided into 4 groups.

In the control group (C, n = 18), the rats received saline solution: 1 ml/kg/day intraperitoneally (i.p.) for 15 days; from day -5 (D-5) to day 9 (D9, Fig. 1).

In the doxorubicin-treated group (DOX, n = 18), rats were injected i.p with 1 mg/kg/day doxorubicin (Adriamycin®, Pfizer, Paris, France) for 10 days, from day 0 (D0) to D9. A total dose of 10 mg/kg doxorubicin was administered over the 10-day period (Fig. 1).

In the lipoic acid-treated group (AL), the rats were injected i.p. with 50 mg/kg/day alpha-lipoic acid (Thiogamma 600®, Wörwarg Pharma, Germany) for 15 days, from D-5 to D9. A total dose of 750 mg/kg lipoic acid was administered over the 15-day period (Fig. 1).

In the last group (DOX-AL, n = 18), the rats were treated with both doxorubicin and alpha-lipoic acid at the same dose as the two previous groups. During the first 5 days, the rats were injected with alpha-lipoic acid (50 mg/kg/day), then given a combined therapy: doxorubicin (1 mg/kg/day) and alpha-lipoic acid for 10 days (Fig. 1).

The rats were killed one month (D40; Series I, n = 36) or two months (D70; Series II, n = 32) after the end of the treatment. Each series was composed of 4 groups of animals (C: n = 9; DOX: n = 9; AL: n = 9; DOX-AL: n = 9) (Fig. 1).

The body weight of the rats was monitored daily during the period of treatment (D-5 to D9), then every 10 days. Moreover, food and water consumption was measured. The concentrations of hematocrit (Ht) and plasma hydroperoxide were evaluated throughout the study period.

At the end of the study (D40; D70) the volume of abdominal ascites was measured and the functional parameters of the hearts were evaluated *in vivo* by left ventricular catheterization. Liver tissue samples were harvested for further histological analysis.

2.2. Heart functional parameters measured in vivo

The rats were anaesthetized with sodium thiopental (60 mg/kg, i.p.) and heparinized (500 IU/kg). After thorax depilation, a catheter connected to a pressure transducer, was inserted into the left ventricle through the chest wall in order to measure heart rate (HR) and left ventricular pressures (left ventricular end-diastolic pressure: LVEDP, left ventricular systolic pressure: LVSP, left ventricular developed pressure: LVDP = LVSP-LVEDP) during the first minute of cardiac catheterization. Left ventricle contractility was expressed as +dP/dt and left ventricle relaxation as –dP/dt.

At the end of the study the blood was taken by cardiac puncture, centrifuged and the plasma was immediately frozen in liquid nitrogen. The hearts were excised, cut into segments and frozen.

2.3. Blood/plasma measurements

2.3.1. Hematocrit (Ht)

The hematocrit (Ht, or erythrocyte volume fraction) of the rats' blood was measured before the injection of doxorubicin or alpha-lipoic acid (D-5), at the end of the treatment (D10) and every 15 days during period of observation. For this purpose, a small quantity of blood was collected from

the tail in a heparinized capillary tube and centrifuged to determine the proportion of blood volume made up by red blood cells.

2.3.2. Plasma lipid peroxidation

Determination of plasma hydroperoxides using the "FORT" test.

The "FORT" test is a colorimetric test based on the ability of transition metals such as iron, to catalyze in the presence of hydroperoxides (ROOH), the formation of free radicals (ex: RO•, ROO•) which are then trapped by a chemical amine derivative ($CrNH_2$). This amine reacts with free radicals to form a stable colored cation radical ($CrNH_2^{\bullet+}$) the absorbance of which is measured at 505 nm. The intensity of the color correlates directly with the amount of free radical compound (Beer Lambert Law) and, consequently, the oxidative level of the sample analyzed [33].

Hydroperoxides were measured in the plasma at D-5, D0, D10, D40 and D70.

Determination of Thiobarbituric Acid Reactive Substances (TBARs).

Plasma lipid peroxides were measured by a colorimetric reaction with thiobarbituric acid. One point five ml of trichloroacetic acid/thiobarbituric acid/hydrochloric acid solution was added to 500 µL of plasma. The color of the thiobarbituric acid pigment was developed in a water bath at 100°C for 15 mn. After cooling with ice to room temperature, 1 ml of 70% trichloroacetic acid was added. After 1h 30, the tubes were centrifuged and the color of the TBARs layers was measured at 553 nm. The absorbance values were compared with a standard curve. Results are expressed in µmoles/g proteins; plasma protein was determined according to the method of Bradford [29]

Plasma TBARs were assessed only at the end of the study (D40; D70).

2.3.3. Plasma ascorbyl free radical (AFR) assessment by electron spin resonance (ESR) spectroscopy

35 µL of plasma samples were inserted into a quartz capillary tube which was placed in a HS cavity in order to analyze them at room temperature with a Bruker EMX-100 X-band spectrometer (Wissembourg, France). The following parameters were selected for optimal detection of AFR [34]: modulation frequency: 100 kHz, amplitude modulation: 0.8 G, microwave

power: 40 mW, microwave frequency: 8.5 GHz, conversion time: 40 ms, time constant: 327 ms, scan time: 41 s, gain: 5.10^5 , number of scans: 6. The height of AFR signal intensity was measured and expressed in arbitrary units (AU).

2.4. Measurements in tissues

2.4.1. Measurement of cardiac Thiobarbituric Acid Reactive Substances (TBARs)

Heart lipid peroxides were measured by a colorimetric reaction with thiobarbituric acid. Hearts were homogenized in ice-cold phosphate buffered saline (0.05 M, pH 7). Then, 1.5 ml of trichloroacetic acid/thiobarbituric acid/hydrochloric acid solution was added. The color of thiobarbituric acid pigment was developed in a water bath at 100°C for 15 mn. After cooling to room temperature with ice, 1 ml of 70% trichloroacetic acid was added. After 1h 30, tubes were centrifuged and the color of the TBARs layers was measured at 553 nm. The absorbance values were compared with a standard curve. Results are expressed in µM/g of cardiac tissue.

2.4.2. Superoxide Production by Fluorescence Histology

In the presence of superoxide, ethidine, a fluorescent compound, is formed from dihydroethidium (DHE) making it possible to quantify superoxide production. Frozen heart tissues were fixed for 10 mn in acetone. Slides were incubated in a light-protected humidified chamber at room temperature with DHE (5 µM) for 5 mn. The slides were immediately analyzed with a computer-based digitizing image system (Microvision, France) using a fluorescent microscope (Eclipse 600, Nikon, France) connected to a video camera (TriCCD, Sony, France). Fluorescence was detected at 590 nm and carried forward to the nuclear number. Results are expressed in fluorescence intensity/nuclear number.

2.5. Statistical analysis

All data are expressed as means ± S.E.M. To compare the groups, at 1 month and at 2 months after the end of the treatment, statistical analyses were performed with the one-factor analysis of variance (ANOVA) test (SigmaStat); ANOVA was followed, if necessary, by a Newman Keuls test. To compare the evolution of parameters throughout the period of the study (period of treatment and period of observation) we used a two-factor repeated measures analysis of variance (ANOVA) test (SigmaStat). Significance was established at a value of $P < 0.05$.

3. Results

Two rats of the DOX-AL group died during the observational period (at day 26 and day 30 after the end of the treatment). A very large volume of abdominal ascitic fluid was observed in these dead rats.

3.1. Body weight and food consumption in rats

Both controls and lipoic acid-treated rats gained weight during the period of study (period of treatment and period of observation). Doxorubicin induced a significant loss of body weight starting from the 3rd day of treatment. Lipoic acid associated with doxorubicin treatment was not able to counter this body weight loss. After interruption of the treatment, there was a trend towards recovery of body weight in rats from the DOX and DOX-AL groups with kinetics identical to those of rats from the Control and AL groups (Fig. 2A).

In our study, as might be expected, we found that body weight and food consumption followed approximately the same trend (Fig. 2B). Regarding the quantity of water drunk, no significant differences were observed among the four groups of rats except during the 1st and 4th day after the end of the treatment when those treated with doxorubicin drank a smaller quantity of water than did the control rats (data not shown).

Two months after the end of the treatment (D70) body weight recovery was greater in DOX-AL rats than in DOX rats, but without an increase in food consumption (Fig. 2A, 2B). However, it appeared later that body weight gain in the DOX-AL group was due to the presence of a larger volume of abdominal ascitic fluid (Table 1).

3.2. Abdominal ascitic fluid and liver histological analysis

We noted that the accumulated volume of ascitic fluid at 1 month (D40) and 2 months (D70) after the end of the treatment was significantly higher in rats treated with doxorubicin alone or combined with alpha-lipoic acid (DOX -AL). It was also observed that the quantity of abdominal ascitic fluid was greater in the DOX-AL group than in the DOX group (Table 1). Histological analysis showed the presence of condensation and sinusoidal dilation in liver tissues of rats treated with doxorubicin alone or in combination with lipoic acid (Table 2) but the transaminase plasma levels (ASAT and ALAT) were not changed (data not shown).

3.3. Heart weight, heart weight to body weight ratio

Two months (D70) after the end of the treatment, doxorubicin alone or associated with alpha-lipoic acid induced a significant decrease in heart weight as compared to that of control or AL groups (Fig. 3A). The heart to body weight ratio, which estimates cardiac hypertrophy, was significantly ($P<0.05$) increased in the DOX group (Fig. 3B).

3.4. Cardiac parameters evaluated in vivo by left ventricle catheterization

Heart rate (HR) was not influenced by doxorubicin treatment at D40 and D70. Left ventricular developed pressure (LVDP), +dP/dt and –dP/dt were not modified 1 month after the end of treatment (D40) but at D70, we observed in animals treated with doxorubicin alone (DOX) or in combination with alpha-lipoic acid (DOX-AL) a significant decrease ($P = 0.034$) in +dP/dT (Table 3).

3.5. Blood/plasma parameters

3.5.1. Hematocrit (Ht)

As shown in Figure 4, a cumulative dose of 10 mg/kg of doxorubicin over10 days (D10) induced a decrease in Ht values and the emergence of anemia in rats of the DOX and DOX-AL groups. This anemia persisted for 15 days (D25) after the end of the treatment. Subsequently, during the period of surveillance, Ht values were similar in the four groups of rats.

3.5.2. Plasma hydroperoxide (ROOH) concentration determined by the "FORT" test

Ten days of treatment with alpha-lipoic acid (50 mg/kg/day) induced a significant decrease in plasma hydroperoxide levels in the AL group as compared with the control group: (1.36 ± 0.08 vs. 2.44 ± 0.41 mmol/l H_2O_2, $P<0.05$), which was noticeable only after 5 days of treatment in both AL-treated groups. A cumulative dose of 10 mg/kg of doxorubicin led to an increase in ROOH in both DOX and DOX-AL groups in comparison with the AL group. One month after the end of the treatment (D40), levels of plasma hydroperoxides remained higher in animals treated with doxorubicin. This was no longer the case 2 months after the end of the treatment (Fig. 5).

3.5.3. Plasma concentration of Thiobarbituric Acid Reactive Substances (TBARs)

Two months after the end of the treatment, plasma levels of TBARs were significantly higher in the DOX-AL group than in the Control and AL groups; while only a tendency (P = 0.059) to higher TBARs values was observed in the DOX group (Table 4).

3.5.4. Ascorbate plasma concentrations

One month after the end of the treatment, the plasma concentration of ascorbate was significantly lower in the DOX-AL group than in the AL and Control groups. Two months after the end of the treatment (D70) the decrease in plasma concentrations of ascorbate was more evident in both groups of rats treated with doxorubicin: DOX and DOX-AL and associated with a significant increase in the AFR to ascorbate ratio (Table 4).

3.6. Tissue parameters

3.6.1. Heart tissue TBARs

Two months after the end of the treatment, there was a significant (+50%, $P<0.01$) increase in TBARs in the hearts of rats from the DOX and DOX-AL groups (Table 3) compared with the values in tissue samples of C or LA hearts.

3.6.2. Heart tissue superoxide anion production

The production of superoxide anion ($O_2^{\bullet -}$) assessed with DHE, revealed a higher oxidative stress in the cardiac tissues of rats treated with doxorubicin alone (DOX) 1 month after the end of the treatment. Two months after the end of the treatment, $O_2^{\bullet -}$ production in the heart tissue was significantly higher in both groups of rats treated with doxorubicin (DOX, 9.10 ± 0.18 and DOX-AL, 9.40 ± 0.19) than in the Control (4.60 ± 0.10) and AL (4.70 ± 0.11) groups (Table 4).

4. Discussion

The administration of a cumulative dose of 10 mg/kg doxorubicin induced a decrease in rats' body weight, associated with reduced food consumption. After the end of the treatment, during the two months of the experimental design, body weight and ingested food recovered but did not reach values of the Control group. Treatment with alpha-lipoic acid had no effect on doxorubicin-induced anorexia. It is noteworthy that for rats treated with doxorubicin in combination with alpha-lipoic acid the greater weight recovery was not due to food ingestion but was secondary to a larger accumulation of ascitic fluid, which was revealed and measured at the time the rats were killed. The presence of ascitic fluid could be the consequence of impaired cardiac, renal or liver function [35-36]. In our situation, the cardiac alterations were more obvious, as revealed by an alteration in heart contractility.

Besides anorexia, doxorubicin induced anemia that persisted for 15 days after the end of the treatment; subsequently, the hematocrit values returned to normal. Anemia is a frequently reported side-effect of chemotherapy.

Doxorubicin alone or combined with alpha-lipoic acid induced a decrease in heart weight 2 months after the end of treatment. This happened sooner in rats treated with doxorubicin alone. This phenomenon has been described in other studies [37-38] and could be explained by cardiomyocyte apoptosis [39-41]. The heart to body weight ratio, an index of cardiac hypertrophy, was increased 2 months after treatment in DOX group. In the DOX-AL group this ratio was not significantly altered, probably because of the "artificial" increase in body weight due to the accumulation of ascites.

Cardiac functional parameters, measured in vivo in our experimental conditions, were affected 2 months after treatment with a cumulative dose of 10 mg/kg doxorubicin, with lower left ventricular contractility (+dP/dt) in DOX and DOW-AL hearts, inducing a non significant reduction in PDVG and in the left ventricular relaxation index ($-dP/dt$). As has already been observed in our laboratory [42], the impairment of contractility is a very late event and is only at its beginning two months after a cumulative dose of 10 mg/kg, while the general condition of the rats is deteriorating so dramatically that it is life threatening and leads to high mortality. Sacco et al [37] showed that a total cumulative dose of 7.5 mg doxorubicin did not affect PDVG but decreased +dP/dt 13 weeks after the end of treatment. In fact, deterioration in heart contractility could become more evident after cardiac β-adrenergic stimulation with isoprenaline [37].

In our experimental conditions, the addition of alpha-lipoic acid to doxorubicin treatment did not prevent either cardiac alterations or a deterioration in the general physical condition. Al-Majed et al [43] noted a cardioprotective effect of alpha-lipoic acid 48 hours after doxorubicin administration. While alpha-lipoic acid combined with doxorubicin might provide some protection for a few days after doxorubicin treatment, there are no studies to confirm or reject this cardioprotective effect in the long term after the end of chemotherapy.

Regarding the assessment of plasma and cardiac oxidative stress, the methods used in this work were various and complex, each with different characteristics of sensitivity and specificity. Plasma lipid peroxidation was assessed as 1) plasma hydroperoxide concentration evaluated during the treatment and at the moment the rats were killed and 2) plasma malonedialdehyde (MDA) concentration measured at the time the rats were killed (1 and 2 months after the end of treatment). We noted a significant decrease in plasma levels of hydroperoxide after a cumulative dose of 250 mg/kg alpha-lipoic acid (5 days of treatment). This decrease was maintained until the end of treatment (15 days of treatment with a cumulative dose of 750 mg/kg alpha-lipoic acid) with no evidence of a dose-response relationship. In addition, a cumulative dose of 10 mg/kg doxorubicin (10 days of treatment) induced an increase in plasma hydroperoxides; the values were 2.5 times higher than those in the AL group. These results reflect early stress due to circulating free radicals during doxorubicin treatment. On the other hand, 2 months after the end of treatment, plasma TBARs were significantly higher in rats treated with doxorubicin in combination with alpha-lipoic acid (DOX-AL group).

To get a better evaluation of plasma oxidative stress, it was necessary to evaluate the levels of some antioxidant systems. Thus, we determined the plasma concentration of vitamin C by HPLC and the plasma ascorbyl radical level by electron paramagnetic resonance (EPR) spectroscopy. Two months after treatment, the plasma concentration of ascorbate was significantly lower in rats treated with doxorubicin alone or in combination with alpha-lipoic acid. This phenomenon can be explained by a decrease in hepatic vitamin C synthesis and by an excess of free radical species production as we previously reported [38].

Evaluation of cardiac oxidative stress by TBARs or by ethidine (DHE) fluorescence showed a significant increase in $O_2^{\bullet -}$ production and in malonedialdehyde levels in the DOX and DOX-AL groups, 2 months after doxorubicin administration. In a similar study carried out in our laboratory, cardiac oxidative stress was not evident 8 days after treatment with a cummulative

dose of 10 mg/kg doxorubicin [38]. This could confirm that tissue oxidative stress is a late event in the onset of doxorubicin cardiotoxicity.

It is important to note that, in our experimental conditions, that oxidative stress in the plasma or cardiac tissue was not improved by the addition of alpha-lipoic acid to the doxorubicin treatment. Despite this, it is important to remember that alpha-lipoic acid alone significantly decreases the concentration of plasma hydroperoxides, but in combination with doxorubicin it had no beneficial effect. Contrary to our initial hypothesis, the results obtained regarding mortality and the presence of high quantities of ascites indicate that the combination of doxorubicin with alpha-lipoic acid is rather deleterious than beneficial to the parameters evaluated.

In contrast to our results, some experimental studies showed that alpha-lipoic acid administered 24 h before the injection of doxorubicin, once a week over a period of 10 to 12 weeks, had a beneficial effect in renal [44-45] and testicular toxicity [46], but did not mention major side-effects. The ineffectiveness of alpha-lipoic acid in preventing doxorubicin cardiotoxicity found in our study could be due to the inhibition of compensatory mechanisms activated at the start of chemotherapy. Indeed, there is a long period of time during which there are no modifications in oxidative stress status or in cardiac contractile parameters. This latent period without symptoms might be secondary to the involvement of compensatory mechanisms that are triggered quite early [35, 47]. It is not easy to evaluate the specific pharmacologic actions of an antioxidant. For instance, it is known that antioxidants can prevent the cardioprotective mechanisms triggered by ischemic preconditioning, whereas the administration of a free radical-generating system may mimic ischemic preconditioning [48-50]. It is worth mentioning, though, that in our protocol, the alpha-lipoic acid was administered 5 days prior to the introduction of doxorubicin. The reactive oxygen or nitrogen species formed after the first doses of anthracyclines might induce cellular protective pathways that are unfortunately overwhelmed later by the following administrations of anthracycline. Then, pre-treatment with an antioxidant such as alpha-lipoic acid might paradoxically reinforce the toxic effects of doxorubicin, not only for the oxidative stress levels but also for cellular injury. In addition, a possible negative interaction between the two substances cannot be excluded. Further work is obviously needed to confirm or rule out these hypotheses, and to explore more thoroughly the timing of doxorubicin and alpha-lipoic acid administration or the dosages used.

5. Conclusion

In conclusion, this study confirmed that treatment with doxorubicin quickly deteriorated the general state of the animals while the cardiac problems and oxidative stress in the plasma and tissue were more obvious two months after completion of the treatment. Contrary to our initial hypothesis, the administration of alpha-lipoic acid in combination with doxorubicin did not have a beneficial effect on heart function or on general oxidative stress, and led to a greater accumulation of ascitic fluid. The “beneficial” antioxidant properties of alpha-lipoic acid clearly appear to depend on the experimental conditions, and the cardioprotective potential of some antioxidant molecules in the context of chemotherapy should be approached with caution.

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Legends to figures:

Fig. 1: Experimental protocol. In the control group (C, n = 18), rats received saline solution: 1 ml/kg/day intraperitoneally (i.p.) for 15 days; from day -5 (D-5) to day 9. In the doxorubicin-treated group (DOX, n = 18), rats were injected i.p with 1 mg/kg/day doxorubicin for 10 days, from day D0 to D9. In the lipoic acid-treated group (AL), rats were injected i.p. with 50 mg/kg/day alpha-lipoic acid for 15 days, from D-5 to D9. In the last group (DOX-AL, n = 18), rats were treated with both doxorubicin and alpha-lipoic acid at the same dose as the two previous groups. The rats were killed one month (D40) or two months (D70) after the end of the treatment with doxorubicin or saline, and hematocrit (Ht) was periodically measured.

Fig. 2: Evolution of body weight [A] and food consumption [B] in the control group (C), alpha-lipoic acid group (AL), doxorubicin group (DOX) and doxorubicin + alpha-lipoic acid group (DOX-AL) throughout the period of study (**P<0.01: DOX and DOX-AL *vs.* C and AL; $^{\&\&}$P<0.01: DOX *vs.* C and AL; †P<0.05: DOX-AL *vs.* C and AL; $^{£}$P<0.05 $^{££}$P<0.01: DOX-AL *vs.* DOX).

Fig. 3: Heart weight [A] and heart to body weight ratio [B] in the control group (C), alpha-lipoic acid group (AL), doxorubicin group (DOX) and doxorubicin + alpha-lipoic acid group (DOX-AL), 2 months (D70) after the end of the treatment.

A: P<0.01: DOX and DOX-AL *vs.* C and AL

B: P<0.05: DOX *vs.* C and P<0.05: DOX-AL *vs.* DOX

Fig. 4: Evolution of hematocrit throughout the period of study, in the control group (C), alpha-lipoic acid group (AL), doxorubicin group (DOX) and doxorubicin + alpha-lipoic acid group (DOX-AL) ($^{*}P<0.05$, $^{**}P<0.01$: DOX and DOX-AL *vs.* C and AL).

Fig. 5: Evolution of plasma hydroperoxides (ROOH) throughout the period of study in the control group (C), alpha-lipoic acid group (AL), doxorubicin group (DOX) and doxorubicin + alpha-lipoic acid group (DOX-AL).