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Adriamycin-related anxiety-like behavior, brain oxidative stress and myelotoxicity in male Wistar rats

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ABSTRACT

Chemotherapeutic regimens have been indicated to negatively impact the quality of life for patients. Adriamycin (ADR) is an effective chemotherapeutic agent widely employed for the treatment of human's malignancies; however, it may cause serious side effects. The present study was aimed at investigating the effects of acute administration of ADR on cognitive alterations, brain oxidative status and immune dysregulation in male Wistar rats. Treated animals received a single intraperitoneal injection of ADR (7 mg/kg). Control ones received physiological saline only. Behavioral effects were tested in the elevated plus-maze and the open field which showed that drug-treated rats displayed anxious behavior and deteriorations in the locomotive and exploratory activities over the 72 h following ADR injection as compared to controls. Assessment of brain antioxidant capacity in ADR-injected animals revealed an increase in glutathione-S-transferase activities and malondialdehyde levels while a decrease in glutathione concentrations when compared with the vehicle-treated group. Our results indicated that ADR administration decreased total leukocyte, lymphocyte and granulocyte counts, while enhanced monocyte levels. Moreover, white blood cells (WBC) relative counts in ADR-treated rats showed a significant increase in monocytes and granulocytes and a decrease in lymphocytes as compared to controls. This study suggests that ADR-related cognitive impairments are associated with brain oxidative stress and myelosuppression.

1. Introduction

Chemotherapy is considered as a systemic treatment, in which chemical agents are used to destroy cancer cells or to stop them from growing. Although chemotherapy exerts preferential cytotoxic effects on malignant cells, it often affects healthy ones as well (Ménard et al., 2008). This undesired result is referred to as side effects that include myelosuppression, hair loss, fatigue, nausea, peripheral neuropathy and cognitive impairment, which may be acute, chronic or permanent (Meyers et al., 2005). Particularly, chemotherapy-induced cognitive deficits are related to peripheral and/or central neurotoxicity and the neuropsychological disorders, recognized in adults who receive chemotherapy, are labeled as "chemobrain" or "chemofog" (Weiss, 2008).

Among chemotherapeutic drugs, adriamycin (ADR) is an antineoplastic anthracycline commonly used in the treatment of human malignancies including solid tumors and malignant hematological diseases (Quiles et al., 2002). Its anticancer mechanism is attributed to the intercalation into the deoxyribonucleic acid (DNA) double helix, inhibition of nucleic acid synthesis and topoisomerase II activity (Cutts et al., 2003). In spite of this potent antitumor action, the clinical use of ADR is limited by the concomitant normal tissue injuries, such as cardiomyopathy (Sacco et al., 2003).

Recently, chemotherapy-related cognitive impairment has been reported in patients who receive cancer therapy not only for the brain but also for peripheral locales such as the breast (Jansen et al., 2008; Morse et al., 2003). These persistent cognitive changes frequently resulted in a decrease of information processing speed, motor and executive dysfunctions, visual and verbal memory loss, attention and concentration deficits and spatial skill impairment (Ahles and Saykin, 2007; Jansen et al., 2008; Tannock et al., 2004). In this respect, adriamycin-based chemotherapy of advanced malignant diseases, especially in breast cancer patients, has shown consistent cognitive problems (Freeman and Broshek, 2002).

Since clinical determination of these cognitive deficits is difficult, several studies in established rodent models have been reported

Abbreviations: ADR, adriamycin; ANOVA, analysis of variance; EPM, elevated plus-maze; HPA axis, hypothalamo–pituitary–adrenal axis; OF, open field.

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(ELBeltagy et al., 2010; Liedke et al., 2009; Macleod et al., 2007; Mustafa et al., 2008). These experimental studies, in which animals were exposed to single or combined chemotherapeutic drugs, have tested rodents' performance in multiple tasks such as Morris water maze (Lee et al., 2006; Seigers et al., 2008), Stone 14-unit T-maze, (Lee et al., 2006), inhibitory avoidance (Liedke et al., 2009), open field (Konat et al., 2008; Liedke et al., 2009), contextual conditioned response (ELBeltagy et al., 2010), fear conditioning (Macleod et al., 2007) and object location recognition (ELBeltagy et al., 2010; Mustafa et al., 2008). A great part of these animal studies have found a pronounced cognitive impairment following chemotherapy treatment (Konat et al., 2008; Mustafa et al., 2008). However, some reports have shown no effect or an improvement in cognition (Gandal et al., 2008; Lee et al., 2006). Interestingly, ADR-induced memory impairment has been approved in both humans and animals when the molecule is used either alone or in combination with other agents such as cyclophosphamide (Jansen et al., 2008; Liedke et al., 2009; Macleod et al., 2007).

Recent studies have revealed that brain tissue is highly susceptible to chemotherapeutic drugs (Ahles and Saykin, 2007; Chen et al., 2007; Oboh and Ogunruku, 2010). Although ADR is unable of crossing an intact blood–brain barrier (Ohnishi et al., 1995), it can potentiate plasma and brain levels of cytokines such as tumor necrosis factor (TNF) alpha (Tangpong et al., 2006; Usta et al., 2004). These mechanisms have been shown to especially result in brain oxidative stress (Joshi et al., 2005, 2007; Tangpong et al., 2006, 2007), which may predispose subjects to cognitive impairment and neurodegenerative conditions (Cardoso et al., 2008; Dubovický, 2010). Oxidative stress, caused by the increase of free radical generation and/or the impairment of endogenous antioxidant mechanisms, has been implicated in various neurodegenerative diseases (Butterfield and Lauderback, 2002; Moreira et al., 2005). The brain may especially be at risk of free radical-mediated injury because it is characterized by low concentration of antioxidant enzymes and free radical scavengers as well as neuronal membranes that are rich in polyunsaturated fatty acids (Shulman et al., 2004). Interestingly, ADR-generated free radicals in the brain may enhance protein oxidation and lipid peroxidation and lead to neuronal tissue dysfunction and cell death (Joshi et al., 2005, 2007, 2010). Furthermore, Cardoso et al. (2008) revealed that ADR treatment increases the susceptibility of brain mitochondria to oxidative stress and Ca^{2+}-induced permeability transition pore opening. However, the mechanisms by which ADR causes brain oxidative stress are still unclear.

Chemotherapy-associated immunosuppression is due to toxic effects of drugs on immune cells in bone marrow and peripheral lymphoid tissues (Wijermans et al., 1993; Park et al., 2009). Thus, the increase in anthracycline doses has been associated with an elevation in the incidence of myelotoxicity, resulting in a higher incidence of neutropenia (Debled et al., 2007) and lymphopenia (Sfikakis et al., 2005; Tolaney et al., 2008). For example, Branda et al. (2006) reported that increasing doses of adriamycin, injected in female Fisher 344 rats, were paralleled with decreasing levels of hematocrit and total white blood cells at the 4th day post treatment. This chemotherapy-induced myelosuppression might result in release of inflammatory cytokines that cross the blood–brain barrier, leading to cognitive dysfunction and/or fatigue (Ahles and Saykin, 2007; Branks et al., 2003; Meyers et al., 2005). In this respect, several investigations have shown that central nervous (CNS) and immune (IS) systems are intimately linked (Banks and Erickson, 2010; Wrona, 2006). Hence, the CNS can modulate immune responses not only via neurotransmitters, neuropeptides, neurotrophic factors and endocannabinoids (Irani, 2002; Tian et al., 2009), but also following activation of the hypothalamic–pituitary–adrenal (HPA) axis (Haddad et al., 2002; Wrona, 2006) and the sympathetic nervous system (SNS) (Madden, 2003; Wrona, 2006). Conversely, cytokines released by immune cells are believed to directly regulate neuronal function (Engelhardt, 2008; Xiao and Link, 1998), or to stimulate the CNS-derived cytokines production (Adler et al., 2006; Jean-Gilles et al., 2010).

The purpose of this study aims at investigating the effects of a single chemotherapeutic drug, adriamycin, on post-treatment behavioral and hematological changes as well as brain oxidative stress parameters in male Wistar rats.

2. Methods

2.1. Animals and housing

Eighty-three (83) male Wistar rats obtained from Pasteur Institute (Algiers, Algeria) were housed in transparent cages at a constant temperature (23 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 07:30 a.m.). Rats had access to standard rodents chow and tap water ad libitum. Weighing 230–250 g at the beginning of the experiment, the animals were weighed daily before any other experimental procedure in order to calculate the 24 h-body weight gains. The study protocol was carried out according to the NIH revised Guidelines for the Care and Use of Laboratory Animals (no. 80–23, 1996).

2.2. Treatment and experimental groups

Clinically, doses of ADR are administered in the range of 30–70 mg/m^2 of human body surface, which are approximately equivalent to 4–10 mg/kg of body weight respectively in the rat (Food and Drug Administration, 2010). Thus, we have chosen a middle dose of 7 mg/kg of body weight. Rats were either treated with a single intraperitoneal (i.p.) injection of 0.9% saline-dissolved adriamycin (doxorubicin hydrochloride, Sigma-Aldrich Co., Steinheim, Germany) or the same volume of 0.9% saline as control. For this study, two experiments were realized with different groups of rats.

2.2.1. Experiment 1

Thirty-eight (38) rats, housed individually, were divided into two groups (n = 19). Group 1 received saline solution (control) and group 2 received ADR by single i.p. injection. Animals of the two groups were behaviorally tested in the elevated plus-maze and the open field performance tasks 1 h, 24 h, 48 h or 72 h after treatment.

2.2.2. Experiment 2

Forty-five (45) rats were divided into five groups (n = 9) and submitted a single i.p. injection. Group 1 received saline and served as control. Groups 2, 3, 4 and 5 received ADR. Animals were sacrificed by decapitation, under mild diethyl ether anesthesia, 1 h (groups 1 and 2), 24 h (group 3), 48 h (group 4) or 72 h (group 5) after treatment. Blood samples for white blood cells counting were collected into ethylenediaminetetraacetic acid (EDTA)-coated tubes. Animals were immediately dissected and their brains were removed and rinsed with ice-cold isotonic saline. Brains were homogenized with ice-cold 0.1 mol/l phosphate buffer (pH 7.4) to obtain 1:10 (w/v) whole homogenates, which were centrifuged at 10,000 *g* (4 °C) for 15 min. Aliquots of supernatant were separated and used for oxidative stress assessment.

2.3. Behavioral assessment

2.3.1. Open field test

The open field (OF) can be considered as a non-conditioned anxiety test based on the creation of a conflict between the exploratory drive of the rat and its innate fear of exposure to an open area (Angrini et al., 1998). The OF test was performed to measure spontaneous activity in rodents (Sherif and Oreland, 1995). Briefly, the apparatus, as previously described (Sáenz et al., 2006), consisted of a gray square (70 cm x 70 cm x 40 cm) divided into 16 equal squares that had been drawn in the floor of the arena. The test room was dimly illuminated with a red bulb (25 W) located 130 cm above the center of the arena under the same environmental conditions as the colony room. Each rat was placed in the arena individually, and allowed to freely explore for

5 min, while its activities were tracked and recorded using ANYmaze™ computer software (Stoelting Co., USA). Upon completing the task, the rat was removed from the arena by the experimenter and returned to the home cage. After each test, the apparatus was cleaned with an alcoholic solution followed by wet and dry paper towels to avoid transfer of olfactory cues between animals.

2.3.2. Elevated plus-maze test

The elevated plus-maze (EPM) test is a widely used paradigm to investigate anxiety-related behavior in rats (Pellow et al., 1985). The EPM was made of painted wood cross (arms 50 cm long x 10 cm wide) elevated 50 cm above the floor. Two opposite arms were enclosed by walls (10 cm x 50 cm x 45 cm high) and two arms were open. The arms extended from a central platform (10 x 10 cm) (Patin et al., 2005). The open arms in the maze that we use do not have a railing, but addition of a 3–5 mm high railing on the open arms of the plus maze has been used with success to increase open arm exploration. The test room was lit by a 60-W electric bulb hanging directly 175 cm above the central area of the maze (Estanislau and Morato, 2005). The rat was placed in the center of the apparatus facing one of the open arms, for a free exploration of 5 min. Entry into an arm was defined as the animal placing all four paws on the arm. The animal's activities were tracked and recorded by an overhead camera and ANY-maze™ computer software (Stoelting Co., USA). After each test, the rat was returned to its home cage and the maze was cleaned with an alcoholic solution followed by wet and dry paper towels, prior to the next trial.

2.4. Oxidative stress analyses

2.4.1. Glutathione-S-transferase assay

Glutathione-S-transferase (GST) activity was determined according to the method of Habig et al. (1974). In brief, the GST activity toward 1-chloro-2-4-di-nitrobenzene (CDNB) in presence of glutathione as a co-substrate was measured spectrophotometrically. The enzyme activity was determined by monitoring the changes in absorbance at 340 nm and expressed as nmol/min/mg protein.

2.4.2. Reduced glutathione assay

Reduced glutathione (GSH) was estimated according to the method described by Ellman (1959). A 1.0 ml supernatant was precipitated with 1.0 ml of 4% sulphosalicylic acid and cold digested at 4 °C for 1 h. The samples were centrifuged at 1200 *g* for 15 min at 4 °C. The assay mixture contained 1 ml of supernatant, 2.7 ml of 0.1 M phosphate buffer (pH 8.0) and 0.2 ml of DTNB (5-5′dithio-bis-(2-nitrobenzoic acid)). The yellow color developed was read immediately at 412 nm spectrophotometrically. The results were expressed as nmol GSH/mg protein.

2.4.3. Lipid peroxidation assay

As an index of lipid peroxidation, we used the formation of malondialdehyde (MDA) during an acid-heating reaction, which is widely adopted for measurement of lipid redox state, as previously described (Draper and Hadley, 1990). For this purpose, 2.5 ml of 10% trichloroacetic acid (TCA) was added to 0.5 ml of samples in each centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 *g* for 10 min, and 2.0 ml of the supernatant was added to 1.0 ml of 0.67% thiobarbituric acid (TBA) solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex $1.56\times10^5\ cm^{-1}\ M^{-1}$, and was expressed in nmol MDA/mg protein.

2.4.4. Brain protein assay

Protein content in the supernatant was measured using bovine serum albumin as standard, as described previously (Bradford, 1976).

2.5. Hematological analysis

Selected hematological parameters (WBC — total white blood cell count, LYM — Lymphocytes, MONO — Monocytes and GRAN — Granulocytes) were measured using a full automated blood cell counter (PCE-210 model 2009, Japan).

2.6. Statistical analysis

All data are expressed as the mean ± SEM (Standard Error of the Mean). All groups showed normal distributions, so a parametric statistical method; one way analysis of variance (ANOVA) followed by the post-hoc Dunnett's test, when necessary, was used for multiple comparisons. Two way analysis of variance treatment × time was used for behavioral tests. The value of $p<0.05$ or less was considered as the significant difference. Data were analyzed using MINITAB (Minitab® 15.1.1.0., Minitab Inc., USA).

3. Results

3.1. Body weight gain

Intergroup comparisons indicated a significant decrease of body weight gain 24 h, 48 h and 72 h ($p<0.001$) post-ADR compared to control. In fact, ADR-injected rats had a trend of losing more weight 24 h after treatment (data not shown). A two way ANOVA revealed significant effects of treatment ($p<0.001$), time ($p<0.05$) factors and treatment × time interaction ($p<0.01$).

3.2. Anxiety-like behavioral effects of adriamycin in the open field test

Fig. 1(A) illustrates the locomotive distance of the vehicle- and drug-injected animals in the open field. Comparison of individual group means indicated that, compared to saline, ADR decreased the distance traveled in the apparatus at 1 h and 24 h ($p<0.01$), 48 h and 72 h ($p<0.001$). ADR-treated rats show significantly lesser locomotive activity than vehicle-treated rats. Moreover, average and maximum speeds decreased significantly in rats treated with ADR as compared with the control (Fig. 1(C) and (D)). The highest average speed was registered 1 h post-ADR injection. Furthermore, a two way ANOVA on these parameters revealed a significant effect of the treatment ($p<0.001$) but not the time factor nor the treatment × time interaction. In the same way, Fig. 1(B) shows a decrease of rearings in the ADR-treated animals at 1 h, 24 h ($p<0.01$) and at 72 h ($p<0.05$) compared to control. Multiple comparisons using two way ANOVA indicated a significant effect not only of the treatment and time factors ($p<0.001$), but also the treatment × time interaction ($p<0.05$). As shown in Fig. 1(E) and (F), drug-treated rats spent significantly less time in the center area ($p<0.001$), but more time in the peripheral one ($p<0.001$), of the maze than control rats. Two way ANOVA analysis exhibited significant effects of both treatment ($p<0.001$) and time ($p<0.05$) factors, but not the treatment × time interaction.

3.3. Anxiety-like behavioral effects of adriamycin in the elevated plus-maze test

Fig. 2(A) demonstrates that the distance traveled by adriamycin-treated rats in the apparatus decreased significantly as compared with vehicle-treated rats. The two way ANOVA indicated a significant effect of treatment factor ($p<0.001$), but not time factor nor the treatment × time interaction. On the other hand, the results showed that the average speed of drug-treated animals was significantly reduced in comparison with the saline-treated ones (1 h and 24 h: $p<0.01$; 48 h and 72 h: $p<0.001$, Fig. 2(B)). Multiple comparisons using two way ANOVA exhibited significant effects of both treatment ($p<0.001$) and time ($p<0.01$) factors but not the treatment × time interaction.

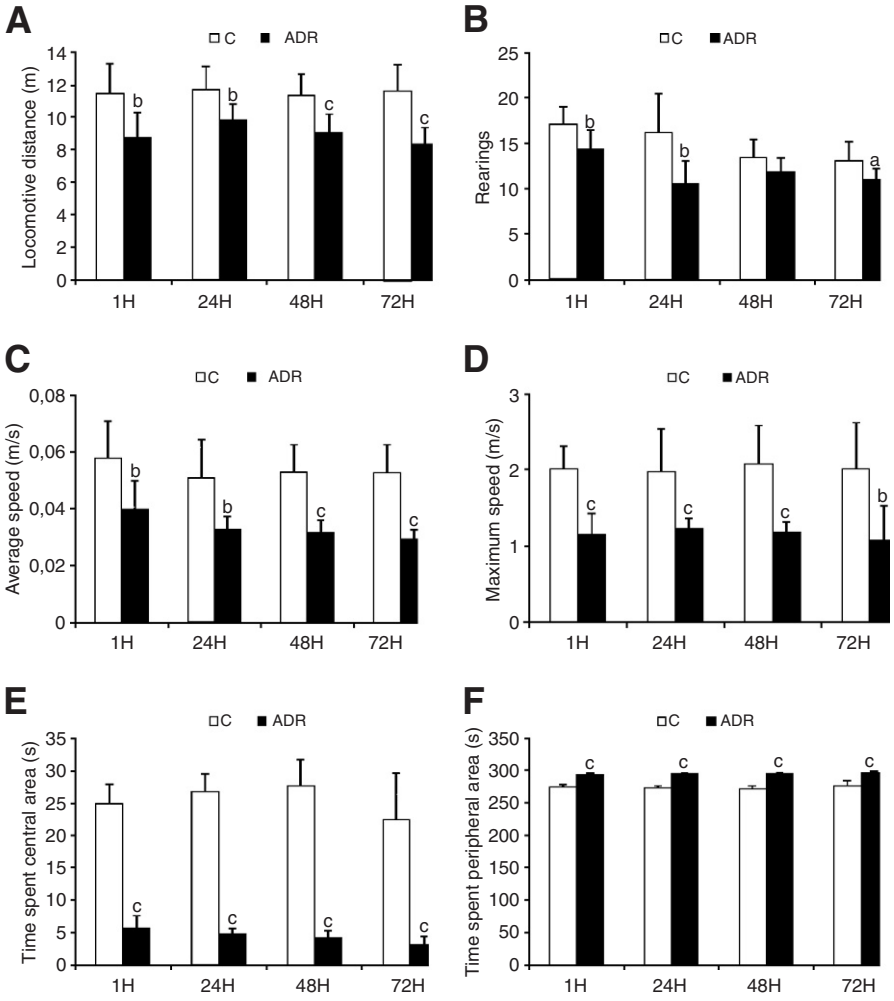

Fig. 1. Open field behavior after adriamycin or vehicle injection. Locomotive distance traveled in the apparatus (A). Number of rearings performed during the test sessions (B). Average and maximum speeds reached in the apparatus (C and D respectively). Time spent in central and peripheral areas (E and F respectively). Data are reported as mean ± SEM (n = 19). C (Control); ADR (Adriamycin). a $p<0.05$, b $p<0.01$, c $p<0.001$, compared to the corresponding control.

Furthermore, Fig. 2(C) illustrated that the maximum speed decreased significantly in rats administered ADR ($p<0.001$) compared to control group. A two way ANOVA analysis indicated significant effects of both treatment factor and treatment × time interaction ($p<0.001$) but not the time factor. Moreover, intergroup comparisons showed that ADR suppressed the number of open arm entries at 1 h ($p<0.01$), at 24 h, 48 h and 72 h ($p<0.001$) compared with saline-treated rats (Fig. 2(D)). The two way ANOVA revealed a significant effect of treatment factor ($p<0.001$), but not time factor nor the treatment × time interaction. In addition, data presented in Fig. 2(F) indicate that ADR treatment reduce the time spent in open arms as compared with the vehicle control group ($p<0.001$). Multiple comparisons using the two way ANOVA showed significant effects of treatment factor ($p<0.001$), time factor ($p<0.05$) and the treatment × time interaction ($p<0.01$).

On the contrary, the numbers of closed arm entries were significantly increased by ADR only at 1 h and 72 h ($p<0.05$) compared with vehicle-treated group (Fig. 2(E)). Two way ANOVA analysis revealed that there were main effects of both treatment and time ($p<0.001$) but not the treatment × time interaction. In the same way, the data collected demonstrate that ADR-treated rats spent significantly more time in the closed arms of the apparatus along the test sessions ($p<0.001$) compared to vehicle-treated rats (Fig. 2(G)). The two way ANOVA analysis exhibited significant effects of both treatment ($p<0.001$) and time ($p<0.01$) factors but not the treatment × time interaction.

3.4. Effects of adriamycin on brain biomarkers

From the results depicted, ADR was found to elicit a significant elevation in GST activity when compared to vehicle-treated group ($p<0.001$, Fig. 3) over the treatment period, but this increase was more registered at 24 h post-ADR exposition.

Fig. 4 shows the modulation of GSH by ADR treatment. In drug-treated rats, there was a significant reduction in the GSH content from controls over the 72-h time after ADR injection ($p<0.001$).

This depletion of antioxidant defense in brain induced by ADR was associated by a significant increase in lipid peroxidation, revealed by the accumulation of MDA in ADR-injected animals as compared to control ones (1 h: $p<0.05$, 24 h: $p<0.01$, 48 h and 72 h: $p<0.001$, Fig. 5).

3.5. Effects of adriamycin on hematological parameters

Total leukocyte counts and WBC relative counts (%) during ADR treatment of rats are presented in Table 1. Compared with the control group, leukocyte count decreased significantly in drug-treated rats (1 h, 24 h and 72 h: $p<0.001$, 48 h: $p<0.01$). The lowest value was registered 24 h post-ADR injection.

Significant lymphopenia ($p<0.001$) was observed in ADR-injected animals when compared to control. The decreased lymphocyte levels were detectable as early as 1 h and were persisted throughout the 72-h period after ADR treatment. In the same way, the percentage of

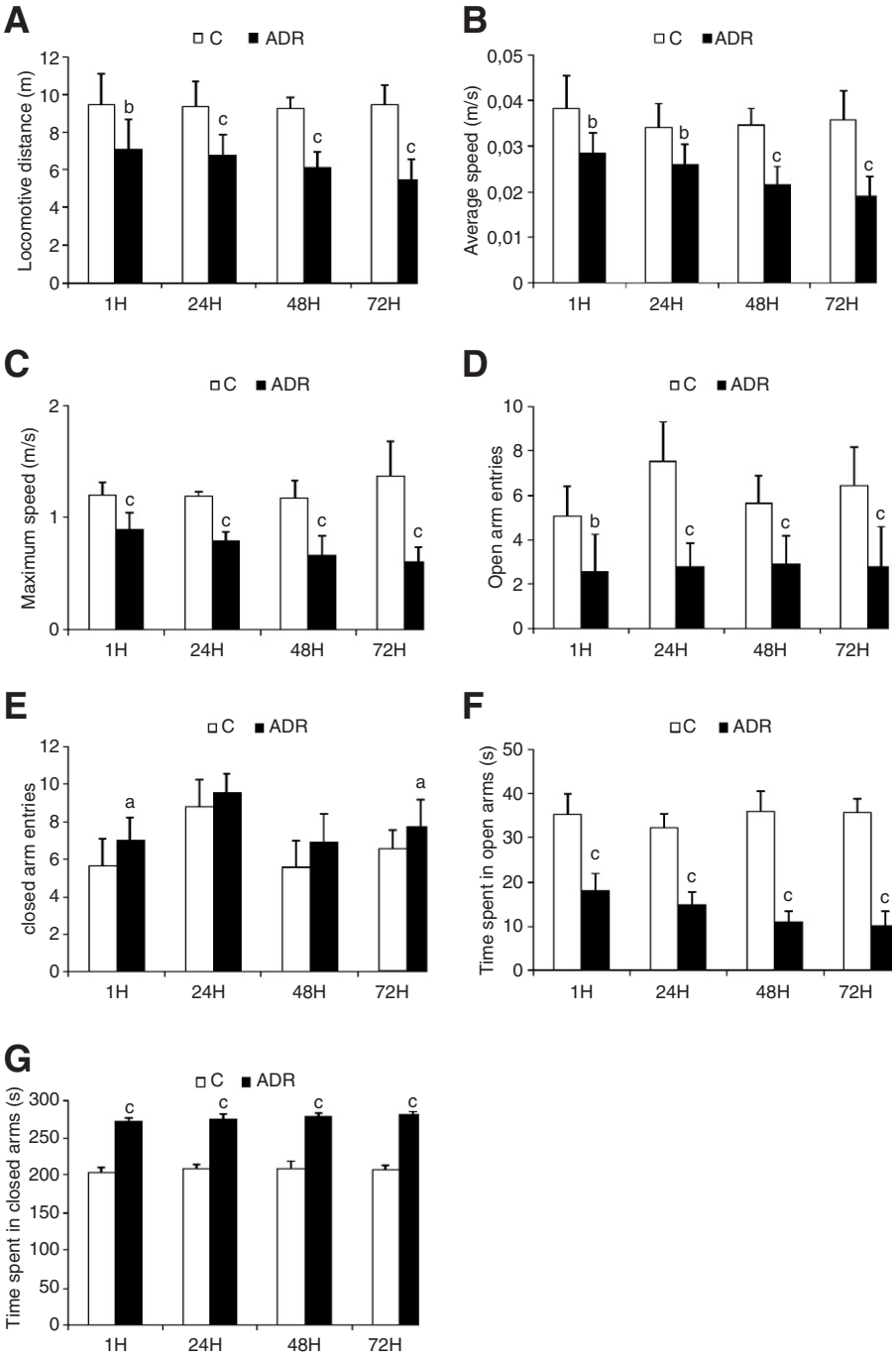

Fig. 2. Elevated plus-maze behavior after adriamycin or vehicle injection. Locomotive distance traveled in the apparatus (A). Average and maximum speeds reached in the apparatus (B and C respectively). Open and closed arms entries (D and E respectively). Time spent in open and closed arms (F and G respectively). Data are reported as mean ± SEM (n = 19). C (Control); ADR (Adriamycin). a $p<0.05$, b $p<0.01$, c $p<0.001$, compared to the corresponding control.

lymphocytes in the total number of leukocytes decreased significantly over the time of exposition ($p<0.001$), but lower at 48 h after ADR injection.

The mean monocyte values increased significantly 1 h, 48 h ($p<0.001$) and 72 h ($p<0.05$) post-ADR as compared to vehicle group, while decreased non-significantly at 24 h. When compared to control, the percentage of monocytes in leukocytes resulted in a statistically significant increase over the 72-h time after ADR injection ($p<0.001$). The highest level was noted as early as 1 h after ADR treatment.

As compared to saline-treated group, the granulocyte levels decrease significantly only at 24 h ($p<0.01$) after drug injection, while increased non-significantly 48 h post-ADR. Furthermore, the relative count of granulocytes in the leukocytes increased significantly in ADR-treated rats ($p<0.001$) at the earliest time point examined (1 h post-ADR) and was maintained over the 72 h of treatment.

4. Discussion

Despite beneficial effects of chemotherapy regimens, recent investigations have reported that they cause neuropsychological disorders including cognitive symptoms, confusion, memory loss and difficulties in attention and concentration (Freeman and Broshek, 2002; Tannock et al., 2004). Actually, chemotherapy-induced cognitive impairments have been revealed in women who receive combination of

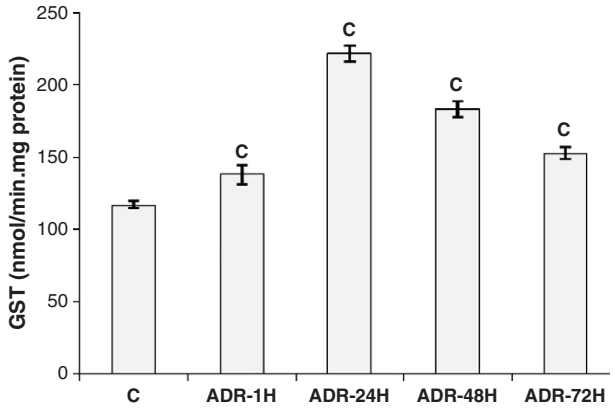

Fig. 3. Effect of adriamycin on glutathione-S-transferase (nmol/min/mg protein) activity in brain of male Wistar rats. Data are reported as mean ± SEM (n = 9). C (Control); ADR (Adriamycin). c $p<0.001$, compared to control.

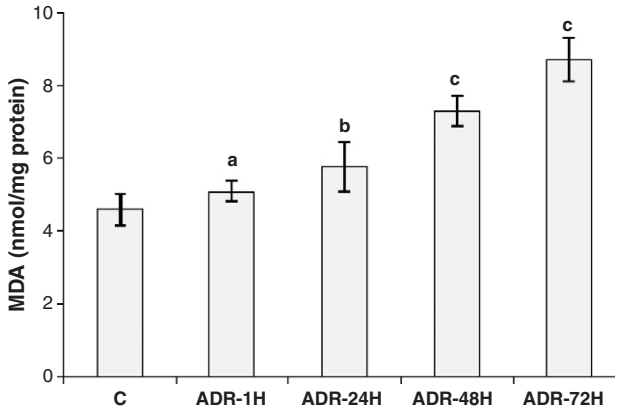

Fig. 5. Effect of adriamycin on malondialdehyde (nmol/mg protein) level in brain of male Wistar rats. Data are reported as mean ± SEM (n = 9). C (Control); ADR (Adriamycin). a $p<0.05$, b $p<0.01$, c $p<0.001$, compared to control.

ADR and cyclophosphamide therapy for breast cancer. These cognitive dysfunctions persisted even after controlling for changes in anxiety, depression, fatigue and perceived cognitive function (Bender et al., 2006; Jansen et al., 2008). Moreover, combined chemotherapy with cyclophosphamide, methotrexate and fluorouracil has shown that the course of general and physical fatigue during and after chemotherapy treatment was significantly different from adriamycin-based chemotherapy. Thus, direct increase in fatigue was seen in ADR group after the start of chemotherapy, whereas the increase in the combined treatment did not show until after the fifth cycle of chemotherapy (De Jong et al., 2004, 2005).

In order to appreciate this somnolence syndrome, collectively referred to as "chemobrain", it was necessary to investigate the effects of chemotherapeutics drugs on animal models. Since it is not known if chemotherapy-related neurotoxicity is caused by the combination of multiple chemotherapeutic agents or by one drug in particular (Morse et al., 2003), we have elicited in the present study, the effects of ADR on locomotor and exploratory activities and anxiety-like behavior in male Wistar rat over a period of 72 h. In the OF test, acute ADR treatment decreased the time spent in the central zone while increased the time spent in the peripheral area, indicating that treated animals prefer staying close to the walls which is commonly known as "Thigmotaxis" (Treit and Fundytus, 1988). A decline of rearings was also seen in ADR-exposed rats, which suggests that this drug interfered with the exploratory behavior (Liedke et al., 2009). In the same way, the EPM test revealed that ADR injection reduced the time spent in and the number of entries into the open arms, while consequently enhanced the time spent in and the number of entries into the closed arms. These findings elicited that drug-treated rats displayed anxious behavior in the two paradigms compared to control animals. Furthermore, we confirmed that ADR decreased the locomotive distance and the average and maximum speeds in both the OF and EPM tasks, suggesting that ADR suppressed the locomotor activity which is an index of the sickness behavior (Konat et al., 2008).

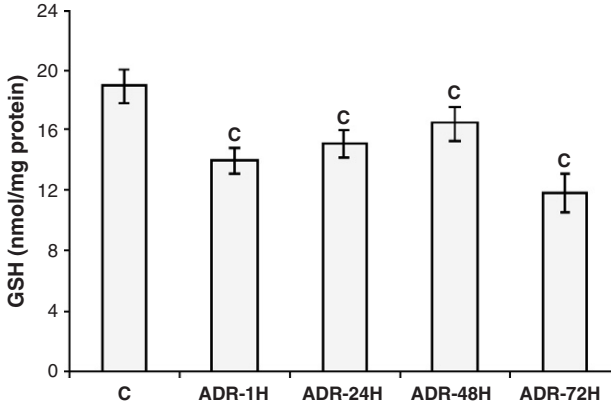

Fig. 4. Effect of adriamycin on reduced glutathione (nmol/mg protein) level in brain of male Wistar rats. Data are reported as mean ± SEM (n = 9). C (Control); ADR (Adriamycin). c $p<0.001$, compared to control.

To our knowledge, ADR-related anxiety-like behavior has not been investigated. Only one study has examined the effects of ADR on locomotor and exploratory activities in male Wistar rat (Liedke et al., 2009). The authors demonstrated that a single i.p. injection of ADR at 8 mg/kg of body weight had no effect on latency to start locomotion, crossings, rearings and number of fecal boli in the OF test, 20 min after treatment. When tested 24 h after ADR injection, the only difference observed was in respect of the number of rearings. These results are not consistent with our findings, except for rearings, probably because of differences in the experimental procedures.

Interestingly, prenatal exposure to different doses of ADR (5, 7 and 9 mg/kg) induced neurobehavioral alterations in 3-month-old C57BL/6J-mice (Van Calsteren et al., 2009). Indeed, the open field test indicated that ADR reduced the total path length and the number of entries in the center with increasing dose, while enhanced the number of entries in the corners. When tested in the elevated plus maze, ADR-exposed mice spent less time in the open arms and elicited signs of hyperlocomotion (i.e. increase of total arm entries). Together, these results indicated changes in emotional behavior and increased anxiety in mice, which are exposed to ADR in utero. These findings corroborate our study, in which male rats were directly treated with ADR.

Since cognitive alterations were identified in women who received chemotherapy regimens for breast cancer or other malignant diseases (Jansen et al., 2008; Morse et al., 2003), recent studies have investigated the effects of different chemotherapeutic drugs on female animal models. As described by Konat et al. (2008), combined chemotherapy regimen with i.p. injections of 25 mg/kg cyclophosphamide and 2.5 mg/kg ADR was administered four times weekly during four weeks in ten-month-old female Sprague Dawley rats. When compared to controls, this chronic treatment elicited no differences in the number of crossings and rearings during 30 min in the OF test, which indicates no effects on locomotor and exploratory activities. On the other hand, the passive avoidance test revealed a profound dysfunction of short-term memory, which was mitigated by a powerful GSH booster, the N-acetyl cysteine (NAC).

Among various groups of antineoplastic drugs, the anthracyclines, such as ADR, generate the highest level of free radicals, leading to oxidative stress in non-targeted organs (Conklin, 2004). In the current study, the brain homogenates of drug-exposed rats showed a persistent increase in GST activity and lipid peroxidation (i.e. increase

Table 1
Blood count parameters after adriamycin or vehicle injection.

Parameters	Control	ADR — 1 h	ADR — 24 h	ADR — 48 h	ADR — 72 h
WBC (x10³/μl)	7.78 ± 0.96	4.16 ± 0.49*	3.40 ± 0.73*	5.60 ± 1.16**	4.33 ± 0.85*
LYM (x10³/μl)	5.36 ± 0.46	1.58 ± 0.24*	1.78 ± 0.64*	1.76 ± 0.36*	1.81 ± 0.53*
MONO (x10³/μl)	0.57 ± 0.11	1.18 ± 0.19*	0.53 ± 0.16	1.41 ± 0.45*	0.77 ± 0.14***
GRAN (x10³/μl)	1.86 ± 0.62	1.38 ± 0.18	1.06 ± 0.21**	2.41 ± 0.56	1.73 ± 0.48
LYM (%)	69.06 ± 3.98	37.87 ± 1.78*	51.23 ± 8.23*	32.16 ± 4.08*	41.80 ± 8.63*
MONO (%)	7.78 ± 2.35	28.80 ± 3.48*	15.78 ± 1.35*	24.98 ± 5.58*	17.90 ± 2.03*
GRAN (%)	23.16 ± 5.71	33.33 ± 2.51*	32.98 ± 8.61***	42.86 ± 3.65*	40.30 ± 7.66*

Data are reported as mean ± SEM for 9 animals per group.
* $p<0.001$ compared to the control group.
** $p<0.01$ compared to the control group.
*** $p<0.05$ compared to the control group.

of MDA level) and depletion in GSH level, indicating that ADR-induced anxiety-like and sickness behaviors are paralleled with a pronounced oxidative stress on neuronal tissues. Increasing amounts of reactive oxygen species (ROS) within the brain resulted in lipid peroxidation, which is revealed through MDA determination (Niki, 2009). In response to these deleterious conditions, the elevation of GST activity may be involved in the protection of CNS against oxidative stress through the transport of toxins away from axons and out of myelin sheaths (Sagara and Sugita, 2001). Importantly, oxidative injury in brain tissues induced GSH depletion (Dringen, 2000), which may be responsible of neuronal cells death (Bains and Shaw, 1997) and neurobehavioral and cognitive deficits in rats (Cruz-Aguado et al., 2001). Our findings confirmed prior results, in which ADR treatment has been shown to cause an alteration in antioxidant enzymatic activity and GSH level resulting in an increase of ROS which induced lipid peroxides generation in neuronal tissues (Joshi et al., 2005, 2007, 2010; Julka et al., 1993). On the other hand, ADR increased nitric oxide which may participate in the CNS toxicity by generating peroxynitrite (Tangpong et al., 2007). Previously, Tangpong et al. (2006) reported that ADR has been detected in the choroid plexus but not in the cortex and hippocampus, indicating that it does not pass the blood–brain barrier. Indeed, ADR enhanced circulating levels of TNF-α, which cross the blood–brain barrier and activate glial cells to further increase local TNF production, leading to reactive nitrogen species (RNS) generation (Chen et al., 2007; Tangpong et al., 2006, 2007). This may support the hypothesis that TNF-mediated ROS and RNS production are involved in ADR-induced brain oxidative stress (Chen et al., 2007), which may lead to chemobrain (Joshi et al., 2005, 2007, 2010).

Most chemotherapy regimens are often considered as myelosuppressants, indicating damage of bone marrow progenitors (Jenkins and Freeman, 2009). Indeed, chemotherapy-induced immunosuppression resulted from depletion of B or T lymphocyte numbers, which may reduce immune response (Steele, 2002). Recent investigations have demonstrated that anthracyclines increase the incidence of myelotoxicity predisposing to life-threatening infections (Debled et al., 2007). In the present study, acute ADR treatment profoundly impacts hematological parameters of the rats. We observed fewer total WBC, a reduction in lymphocyte and granulocyte counts and an elevation of monocytes. The percentage of granulocytes and monocytes in the total number of leukocytes showed an important increase, while the percentage of lymphocytes decreased over the time of exposition. ADR-induced lymphopenia may be resulted from the elimination of lymphocyte precursors and the destruction of mature lymphocyte populations leading to immunodeficiency (Steele, 2002). Monocyte populations have been suggested to be expanded in vivo following cytotoxic antineoplastic therapy which may contribute to T-cell immunosuppression by the production of suppressive factors inhibiting T-cell function (Ageitos et al., 1999; Mackall, 2000). Granulocytopenia following chemotherapeutic regimens increased risk of bacterial, viral, and fungal infections (Tsang et al., 2007). Grant et al. (1991) previously elicited myelo- and immune-suppressive functions of ADR. Thus, three i.p. injections of ADR (cumulative doses: 6 and 12 mg/kg) in female mice dose-dependently depleted the thymus and spleen cells, and the peripheral blood leukocytes and lymphocytes (Pourtier-Manzanedo et al., 1995). Moreover, ADR at 4 mg/kg injected twice a week for a total of 8 i.v. injections in female FVB mice increased blood monocytes 4 days after the fourth injection and 7 days after the last one as compared to saline-treated mice (Asmis et al., 2006). The immune system has been implicated in neurodegenerative diseases that accompany CNS injuries. Interestingly, immunodeficient mice (i.e. severe combined immunodeficiency, SCID) exhibited an impaired cognitive performance in the water morris maze, which was restored following exogenous T cells injection from wild-type mice (Kipnis et al., 2008). These findings support the hypothesis that cognitive deficits could be related to chemotherapy-associated immunosuppression (Ahles and Saykin, 2007; Kipnis et al., 2008; Vardy and Tannock, 2007).

In conclusion, we have demonstrated in this experimental study that ADR, in a single administration, induced anxiety-like behavior and exploratory disorders in male Wistar rats. These chemotherapy-related cognitive dysfunctions were associated with a pronounced brain oxidative stress and an impairment of the immune function, which suggest that both the nervous and the immune systems are intimately linked.

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References

Adler MW, Geller EB, Chen X, Rogers TJ. Viewing chemokines as a third major system of communication in the brain. AAPS J 2006;7:E865–70.
Ageitos AG, Varney ML, Bierman PJ, Vose JM, Warkentin PI, Talmadge JE. Comparison of monocyte-dependent T cell inhibitory activity in GM-CSF vs G-CSF mobilized PSC products. Bone Marrow Transplant 1999;23:63–9.
Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7:192–201.
Angrini M, Leslie JC, Shephard RA. Effects of propanolol, buspirone, pCPA, reserpine, and chlordiazepoxide on open-field behavior. Pharmacol Biochem Behav 1998;59:387–97.
Asmis R, Qiao M, Rossi RR, Cholewa J, Xu L, Asmis LM. Adriamycin promotes macrophage dysfunction in mice. Free Radic Biol Med 2006;41:165–74.
Bains JS, Shaw CA. Neurodegenerative disorders in humans: the role of glutathione in oxidative stress-mediated neuronal death. Brain Res Rev 1997;25:335–58.
Banks WA, Erickson MA. The blood–brain barrier and immune function and dysfunction. Neurobiol Dis 2010;37:26–32.
Banks WA, Farr SA, Morley JE. Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. Neuroimmunomodulation 2003;10(6):319–27.
Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, Paraska KK, et al. Cognitive impairment associated with adjuvant therapy in breast cancer. Psychooncology 2006;15:422–30.
Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
Branda RF, Powden C, Brooks EM, Yildirim Z, Naud SJ, McCormack JJ. Vitamin E but not St. John's wort mitigates leukopenia caused by cancer chemotherapy in rats. Transl Res 2006;148(6):315–24.

Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid-peptide-associated free radical oxidative stress. Free Radic Biol Med 2002;32:1050–60.
Cardoso S, Santos RX, Carvalho C, Correia S, Pereira GC, Pereira SS, et al. Doxorubicin increases the susceptibility of brain mitochondria to Ca^{2+}-induced permeability transition and oxidative damage. Free Radic Biol Med 2008;45:1395–402.
Chen Y, Jungsuwadee P, Vore M, Butterfield DA. St Clair DK. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. Mol Interv 2007;7:147–56.
Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr Cancer Ther 2004;3(4):294–300.
Cruz-Aguado R, Almaguer-Melian W, Díaz CM, Lorigados L, Bergado J. Behavioral and biochemical effects of glutathione depletion in the rat brain. Brain Res Bull 2001;55 (3):327–33.
Cutts SM, Swift LP, Rephaeli A, Nudelman A, Phillips DR. Sequence specificity of adriamycin-DNA adducts in human tumor cells. Mol Cancer Ther 2003;2:661–70.
De Jong N, Candel MJJM, Schouten HC, Abu-Saad HH, Courtens AM. Prevalence and course of fatigue in breast cancer patients receiving adjuvant chemotherapy. Ann Oncol 2004;15:896–905.
De Jong N, Candel MJJM, Schouten HC, Abu-Saad HH, Courtens AM. Course of mental fatigue and motivation in breast cancer patients receiving adjuvant chemotherapy. Ann Oncol 2005;16(3):372–82.
Debled M, Houédé N, Madranges N, Donamaria C, Floquet A, Durand M, et al. Does chemotherapy-induced neutropaenia result in a postponement of adjuvant or neoadjuvant regimens in breast cancer patients? Results of a retrospective analysis. 2007;97:1642–7.
Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990;186:421–31.
Dringen R. Metabolism and functions of glutathione in brain. Prog Neurobiol 2000;62: 649–71.
Dubovický M. Neurobehavioral manifestations of developmental impairment of the brain. Interdiscip Toxicol 2010;3(2):59–67.
ELBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Chur-yoe GT, et al. Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. Behav Brain Res 2010;208(1):112–7.
Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(486):70–7.
Engelhardt B. Immune cell entry into the central nervous system: involvement of adhesion molecules and chemokines. J Neurol Sci 2008;274:23–6.
Estanislau C, Morito S. Prenatal stress produces more behavioral alterations than maternal separation in the elevated plus-maze and in the elevated T-maze. Behav Brain Res 2005;163:70–7.
Food drug administration. FDA dose calculator. Oncology Tools: Dose Calculator, 14/09/2010, 2010, From. http://www.accessdata.fda.gov/scripts/cder/onctools/animalquery.cfm.
Freeman JR, Broshek DK. Assessing cognitive dysfunction in breast cancer: what are the tools? Clin Breast Cancer 2002;3:S91–9.
Gandal MJ, Ehrlichman RS, Rudnick ND, Siegel SJ. A novel electrophysiological model of chemotherapy-induced cognitive impairments in mice. Neuroscience 2008;157: 95–104.
Grant LD, Kaesberg PR, Ershler WB. Immunologic effects of cancer chemotherapy. In: Powis G, Hacker MP, editors. The toxicity of anticancer drugs. New York: Pergamon Press; 1991. p. 167–97.
Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130–9.
Haddad JJ, Saade NE, Safieh-Garabedian B. Cytokines and neuro–immune–endocrine interactions: a role for the hypothalamic–pituitary–adrenal revolving axis. J Neuroimmunol 2002;133:1–19.
Irani D. How much control does the brain exert over the immune system? Curr Opin Neurol 2002;15:323–6.
Jansen CE, Dodd MJ, Miaskowski CA, Dowling GA, Kramer J. Preliminary results of a longitudinal study of changes in cognitive function in breast cancer patients undergoing chemotherapy with doxorubicin and cyclophosphamide. Psychooncology 2008;17:1189–95.
Jean-Gilles L, Gran B, Constantinescu CS. Interaction between cytokines, cannabinoids and the nervous system. Immunobiology 2010;215:606–10.
Jenkins P, Freeman S. Pretreatment haematological laboratory values predict for excessive myelosuppression in patients receiving adjuvant FEC chemotherapy for breast cancer. Ann Oncol 2009;20:34–40.
Joshi G, Sultana R, Tangpong J, Cole MP, St Clair DK, Vore M, et al. Free radical mediated oxidative stress and toxic side effects in brain induced by the anti cancer drug adriamycin: insight into chemobrain. Free Radic Res 2005;39:1147–54.
Joshi G, Hardas S, Sultana R, St Clair DK, Vore M, Butterfield DA. Glutathione elevation by gamma-glutamyl cysteine ethyl ester as a potential therapeutic strategy for preventing oxidative stress in brain mediated by in vivo administration of adriamycin: implication for chemobrain. J Neurosci Res 2007;85:497–503.
Joshi G, Aluise CD, Cole MP, Sultana R, Pierce WM, Vore M, et al. Alterations in brain antioxidant enzymes and redox proteomic identification of oxidized brain proteins induced by the anti-cancer drug adriamycin: implications for oxidative stress-mediated chemobrain. Neuroscience 2010;166(3):796–807.
Julka D, Sandhir R, Gill KD. Adriamycin-induced oxidative stress in rat central nervous system. Biochem Mol Biol Int 1993;29:807–20.
Kipnis J, Derecki NC, Yang C, Scrable H. Immunity and cognition: what do age-related dementia, HIV-dementia and 'chemo-brain' have in common? Trends Immunol 2008;29(10):455–63.
Konat GW, Kraszpulski M, James I, Zhang HT, Abraham J. Cognitive dysfunction induced by chronic administration of common cancer chemotherapeutics in rats. Metab Brain Dis 2008;23:325–33.
Lee GD, Longo DL, Wang Y, Rifkind JM, Abdul-Raman L, Mamczarz JA, et al. Transient improvement in cognitive function and synaptic plasticity in rats following cancer chemotherapy. Clin Cancer Res 2006;12(1):198–205.
Liedke PE, Reolon GK, Kilpp B, Brunetto AL, Roesler R, Schwartsmann G. Systemic administration of doxorubicin impairs aversively motivated memory in rats. Pharmacol Biochem Behav 2009;94(2):239–43.
Mackall CL. T-cell immunodeficiency following cytotoxic antineoplastic therapy: a review. Stem Cells 2000;18:10–8.
MacLeod JE, DeLeo JA, Hickey WF, Ahles TA, Saykin AJ, Bucci DJ. Cancer chemotherapy impairs contextual but not cue-specific fear memory. Behav Brain Res 2007;181: 168–72.
Madden KS. Catecholamines, sympathetic innervation, and immunity. Brain Behav Immun 2003;17:S5–S10.
Ménard C, Martin F, Apetoh L, Bouyer F, Ghiringhelli F. Cancer chemotherapy: not only a direct cytotoxic effect, but also an adjuvant for antitumor immunity. Cancer Immunol Immunother 2008;57:1579–87.
Meyers CA, Albitar M, Estey E. Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer 2005;104(4):788–93.
Moreira PI, Smith MA, Zhu X, Nunomura A, Castellani RJ, Perry G. Oxidative stress and neurodegeneration. Ann N Y Acad Sci 2005;1043:545–52.
Morse R, Rodgers J, Verrill M, Kendell K. Neuropsychological functioning following systemic treatment in women treated for breast cancer: a review. Eur J Cancer 2003;39:2288–97.
Mustafa S, Walker A, Bennett G, Wigmore PM. 5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus. Eur J Neurosci 2008;28:323–30.
Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med 2009;47:469–84.
Oboh G, Ogunruku OO. Cyclophosphamide-induced oxidative stress in brain: protective effect of hot short pepper (*Capsicum frutescens* L. var. abbreviatum). Exp Toxicol Pathol 2010;62:227–33.
Ohnishi T, Tamai I, Sakanaka K, Sakata A, Yamashima T, Yamashita J, et al. In vivo and in vitro evidence for ATP-dependency of P-glycoprotein-mediated efflux of doxorubicin at the blood–brain-barrier. Biochem Pharmacol 1995;49:1541–4.
Park JY, Jang MJ, Chung YH, Kim KY, Kim SS, Lee WB, et al. Doxorubicin enhances $CD4^+$ T-cell immune responses by inducing expression of CD40 ligand and 4-1BB. Int Immunopharmacol 2009;9:1530–9.
Patin V, Lordi B, Vincent A, Caston J. Effects of prenatal stress on anxiety and social interactions in adult rats. Brain Res Dev Brain Res 2005;160:265–74.
Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
Pourtier-Manzanedo A, Didier A, Froidevaux S, Loor F. Lymphotoxicity and myelotoxicity of doxorubicin and SDZ PSC 833 combined chemotherapies for normal mice. Toxicology 1995;99:207–17.
Quiles JL, Huertas JR, Battino M, Mataix J, Ramerez-Tortosa MC. Antioxidant nutrients and adriamycin toxicity. Toxicology 2002;180:79–95.
Sacco G, Giampietro R, Salvatorelli E, Menna P, Bertani N, Graiani G, et al. Chronic cardiotoxicity of anticancer anthracyclines in the rat: role of secondary metabolites and reduced toxicity by a novel anthracycline with impaired metabolite formation and reactivity. Br J Pharmacol 2003;139:641–51.
Sáenz JCB, Villagra OR, Trías JF. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behav Brain Res 2006;169:57–65.
Sagara J, Sugita Y. Characterization of cytosolic glutathione S-transferase in cultured astrocytes. Brain Res 2001;902:190–7.
Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, et al. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behav Brain Res 2008;186(2):168–75.
Sfikakis PP, Gourgoulis GM, Moulopoulos LA, Kouvatseas A, Theofilopoulos AN, Dimopoulos MA. Age-related thymic activity in adults following chemotherapy-induced lymphopenia. Eur J Clin Invest 2005;35(6):380–7.
Sherif F, Oreland L. Effect of GABA-transaminase inhibitor vigabatrin on exploratory behaviour in socially isolated rats. Behav Brain Res 1995;72:135–40.
Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: implications for neuroimaging. Trends Neurosci 2004;27:489–95.
Steele TA. Chemotherapy-induced immunosuppression and reconstitution of immune function. Leuk Res 2002;26:411–4.
Tangpong J, Cole MP, Sultana R, Joshi G, Estus S, Vore M, et al. Adriamycin-induced, TNF-α-mediated central nervous system toxicity. Neurobiol Dis 2006;23:127–39.
Tangpong J, Cole MP, Sultana R, Estus S, Vore M, St Clair W, et al. Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. J Neurochem 2007;100:191–201.
Tannock IF, Ahles TA, Ganz PA, van Dam FS. Cognitive impairment associated with chemotherapy for cancer: report of a workshop. J Clin Oncol 2004;22:2233–9.
Tian L, Rauvala H, Gahmberg CG. Neuronal regulation of immune responses in the central nervous system. Trends Immunol 2009;30(2):91–9.
Tolaney SM, Najita J, Winer EP, Burstein HJ. Lymphopenia associated with adjuvant anthracycline/taxane regimens. Clin Breast Cancer 2008;8(4):352–6.
Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacol Biochem Behav 1988;31(4):959–62.
Tsang YW, Chi KH, Hu CJ, Tseng CL, Tseng FW, Wang YS. Chemotherapy-induced immunosuppression is restored by a fermented soybean extract: a proof of concept clinical trial. Nutr Res 2007;27:679–84.
Usta Y, Ismailoglu UB, Bakkaloglu A, Orhan D, Besbas N, Sahin-Erdemli I, et al. Effects of pentoxifylline in adriamycin-induced renal disease in rats. IPNA 2004;4:1538–45.

Van Calsteren K, Hartmann D, Van Aerschot L, Verbesselt R, Van Bree R, D'Hooge R, et al. Vinblastine and doxorubicin administration to pregnant mice affects brain development and behaviour in the offspring. Neurotoxicology 2009;30:647–57.
Vardy J, Tannock I. Cognitive function after chemotherapy in adults with solid tumours. Crit Rev Oncol Hematol 2007;63:183–202.
Weiss B. Chemobrain: a translational challenge for neurotoxicology. Neurotoxicology 2008;29:891–8.
Wijermans PW, Gerrits WB, Haak HL. Severe immunodeficiency in patients treated with fludarabine monophosphate. Eur J Haematol 1993;50:292–6.
Wrona D. Neural-immune interactions: an integrative view of the bidirectional relationship between the brain and immune systems. J Neuroimmunol 2006;172: 38–58.
Xiao BG, Link H. Immune regulation within the central nervous system. J Neurol Sci 1998;157(1):1–12.