Pakistan J. Zool., vol. 44 (3), pp. 765-776, 2012.

Acute Endotoxemia During Gestation Induces Organ Dysfunction and Tissue Damage in Mouse Offspring

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Abstract.- Sepsis complications represent a systemic inflammatory response to microbial infection or severe injury. This study aimed at determining the effects of septic complications in pregnant female mice on the development of their pups. To achieve this aim, pregnant Swiss mice were given an intraperitoneal injection of endotoxin (lipopolysaccharide, LPS) at a single dose of 2.5 mg/kg body weight. Results revealed that bacterial endotoxin increased oxidative stress in both brain and liver tissue. Malondialdehyde (MDA) was significantly elevated in both LPS-treated mothers and their pups. The anti-oxidant enzyme glutathione was significantly reduced in the LPS-treated mothers and their pups. Free radicals induced by endotoxemic stress mediated the pathological and behavioral complications in the pups born to LPS-treated mothers. Thus, obvious damage was observed in hepatic and intestinal tissues, which in turn led to decreased body weight gain and decrease general activity. Spleen sections from treated mothers and pups showed an increase in trabeculae, high leukocyte infiltration (especially neutrophils and immature megakaryocytes). In contrast, significant depletion of neutrophils was detected in peripheral blood. In addition, pups born to LPS-treated mothers showed significantly lower muscular grip strength than that recorded for pups born to control mothers. The active avoidance test indicated that LPS exposure was associated with learning and memory impairment in the pups. In conclusion, septic complications were actively transferred from endotoxin-treated mothers to developing pups, mediated by high oxidative stress. The oxidative stress, hepatic, ileal, splenic and developmental changes were the major determinants in both mothers and pups.

Key Words: Lipopolysaccharide, mice; offspring, oxidative stress.

INTRODUCTON

The incidence of sepsis and the number of sepsis-related deaths are increasing (Hoesel and Ward, 2004). Septic shock and sequential multiple organ failure have a strong correlation with a high chance of death (Karima *et al.*, 1999). Gram-negative bacteria are the leading cause of septic shock (Remick *et al*., 2000). Bacterial lipopolysaccharide (LPS, also referred to as endotoxin) plays a crucial role in the initiation of host responses to Gram-negative infection. A number of host factors, including cytokines, nitric oxide and eicosanoids, are responsible for mediating most of the manifestations of the endotoxin. Circulatory failure, leukocyte-induced tissue injury and coagulation disorder appear to be critical determinants in the development of sequential organ failure (Karima *et al.,* 1999). Sepsis is associated with multiple organ dysfunction causing disruption of the protective intestinal mucosal barrier and diarrhea containing blood and mucus (Ogunsanya *et al*., 1994). Pathological changes due to sepsis are associated with multiple organ injuries.

Nitric oxide (NO) is generated by three different isoforms of NO synthase, two of which are expressed constitutively (in endothelium: eNOS, brain: nNOS). The third isoform, inducible NOS (iNOS) is induced by LPS or cytokines. Expression of iNOS in many organs or tissues in septic shock results in increased formation of NO that contributes to organ injury and dysfunction, as well as to host defense (Thiemermann, 1997). We have previously found that administration of whole bacteria markedly increased NO synthesis and decreased hepatocyte catalase, total peroxidase and superoxide dismutase activities (Ebaid and Abdel-Salam, 2006). Administration of endotoxin diminished motor

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0030-9923/2012/0003-0765 $ 8.00/0

activity and increased plasma corticosterone and circulating levels of IL-1b, IL-6, TNF-a and IL-10 (Hayley *et al.,* 2008). Because the secretion of these cytokines is induced by LPS and infections, it is possible that they mediate the behavioral responses to infection. Endotoxin is associated with a specific behavioral pattern that includes hypomotility, hypophagia, increased sleep duration, decreased libido, and decreased exploration (Dunn and Swiergiel, 1998).

The gastrointestinal tract acts under normal physiological conditions as a protective barrier against enteric contents such as bacteria and endotoxin, and its integrity may be a critical determinant of clinical outcome in microbial septic patients (Nieuwenhuijzen *et al.*, 1996). We have previously studied ileal injury using two models of microbial sepsis, resulting from either *Aeromonas hydrophila* infection or exposure to its endotoxin. We found that ileal injury was caused by an increased influx of inflammatory and lymphocytic cells associated with hyper-mucus secretion, villar atrophy, necrosis and desquamation (Ahmed and Ebaid, 2008, 2010). Here, after inducing endotoxemia, we followed oxidative stress and sequential organ injuries as critical determinants of septic shock (endotoxemia) in both pregnant female mice and their developing pups to address whether these complications are transferred to the offspring. To achieve this aim, we investigated lipid peroxidation, glutathione levels, liver, spleen and ileal pathological injuries, body weight gain, grip strength and active avoidance learning in mouse mothers and pups. Results proved that all of the septic determinants mentioned above were actively transferred to the offspring, indicating a further dangerous effect of endotoxemia.

MATERIALS AND METHODS

Experimental animals

Male and female Swiss-Webster strain mice (8 weeks old) were housed in opaque plastic cages (three females to one male, or three females without a male, in each cage). Animals were obtained from the Central Animal House, Faculty of Pharmacy, King Saud University. All animal procedures were in accordance with the standards set forth in the guidelines for the care and use of experimental animals by the Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH) protocol. The study protocol was approved by the Animal Ethics Committee of the Zoology Department, College of Science, King Saud University. Animals were kept under reversed lighting conditions with white lights. The ambient temperature was regulated between 18 and 22°C. Food and water were available ad libitum, unless otherwise indicated. The males were removed from the cages after pregnancy was confirmed (appearance of a vaginal plug was considered as day one of pregnancy), and the females were subjected to experimental treatments.

Sepsis model

Females was assigned to one of four groups: 1) the first group was a non-pregnant control group (M) given phosphate buffered saline via an intra-peritoneal injection, 2) the second group was a pregnant control group (M-P) given phosphate buffered saline, 3) the third group was non-pregnant and received a single intra-peritoneal (IP) injection of endotoxin (lipopolysaccharide, LPS) at a dose of 2.5 mg/kg of body weight, 4) the fourth group was pregnant and IP-injected with endotoxin at a dose of 2.5 mg/kg of body weight. Remick *et al.* (2000) have shown that this model demonstrates sepsis-like symptoms, with pathophysiological responses similar to those in patients with sepsis. Mothers and offspring were subjected to biochemical and histological studies. Offspring were subjected to developmental and learning investigations from the day of birth (PD1) until day 21 (PD21).

Blood samples

Animals were anesthetized with pentobarbital (60 mg/kg body weight) and samples (blood and liver) were obtained at the end of the experiment. Whole blood was drawn from the abdominal aorta. Half of the obtained blood was used to evaluate the whole blood and measure the differential count. Heparinized venous blood was centrifuged at 800 × g for 10 min, and plasma was stored at 20°C until analysis.

Glutathione activity associated with endotoxemia

A glutathione (GSH) assay was carried out on

tissue samples according to Clark *et al*. (2010). Briefly, liver and brain of the tested animals were removed and gently rinsed in physiological saline (0.9% NaCl). Fresh weights were recorded and the organs frozen until use. A 10% (w/v) homogenate of each frozen tissue was prepared and the supernatant was used for oxidative stress evaluations. The resulting supernatant was boiled to precipitate and deactivate other proteins, but this did not alter GSH levels (Clark *et al*., 2010; Beutler *et al.*, 1963; Zou *et al.*, 1986). GSH concentrations were then measured by adding 100 µl of boiled supernatant to 400 µl PBS [containing 200 mM MCB and 2U/ml glutathione S-transferase. GSH concentrations were then determined by measuring the absorbance (OD) of the reaction after 1 min at 340nm using a UV Visible Spectrometer (Ultrospec 2000, Pharmacia Biotech). GSH standards were measured concurrently to obtain a standard curve that was used to calculate GSH concentrations in samples. Results were expressed as µg GSH/g tissue. Statistical comparisons of GSH activities between controls and treatments in each case were performed using the Minitab statistical program as detailed below.

Determination of lipid peroxidation

Endogenous lipid peroxidation in tissue homogenates was estimated spectrophotometrically following the method described by Okhawa *et al.* (1979) expressed in nano-moles of malondialdehyde (MDA) per milliliter homogenate (nmole/ml). Tissues were homogenized as described above for the glutathione method. An aliquot of 0.5 ml of the resulting supernatant was shaken with 2.5 ml of 20% trichloroacetic acid (TCA). To the resulting mixture, 1 ml of 0.67% thiobarbituric acid (TBA) was added, shaken, and warmed for 30 min in a boiling water bath followed immediately by rapid cooling in ice for 5 min. After cooling, 4 ml of n-butyl-alcohol was added and the sample shaken well. The resulting mixture was then centrifuged at 16,000 × g for 5 min. The resultant n-butyl-alcohol layer was transferred to a separate tube and MDA content was determined spectrophotometrically at 535 nm using a UV Visible Spectrometer (Ultrospec 2000, Pharmacia Biotech).

Histological sections

Liver, spleen and ileum were collected from the sacrificed control and treated mice. Tissues were fixed in Bouin's fixative, processed in paraffin, and 4 µm thick sections were prepared. Sections were stained with hematoxylin and eosin (H&E) to investigate general histological architecture. In each group, many sections from different mice were studied and representative slides showing clear morphological changes were photographed.

Evaluation of the muscular strength of pups

Thirty days after birth the muscular strength in the fore-feet of pups was measured using a Grip Strength Meter (Ugo Basile, Italy; cat. no. 47105/47106). This apparatus was specifically designed for measuring forelimb grip strength in rodents. Each mouse was placed over a base plate, in front of a T-shaped grasping bar fitted to a force transducer connected to a peak amplifier. When pulled by the tail the mouse grasps at the bar, until the pulling force overcomes its grip strength. The point at which the mouse loses its grip on the grasping bar determined the peak pull-force achieved by the forelimbs.

Learning and memory test in automatic reflex conditioner (shuttle box)

Animals of PD35 were tested using an automated shuttle-box (Ugo Basile, Italy). The shuttle-box was divided into two chambers of equal size with a gate providing access to the adjacent compartment. A 60 W light was switched on for 5 s alternately in the two compartments and used as a conditioned stimulus (CS), which preceded an electric shock (1 mA for 5 s) by 5 s (the unconditioned stimulus). If the animal avoided the unconditioned stimulus by running into the dark compartment within 5 s of the onset of the CS, the shuttle box recorded an avoidance response. Each mouse was given 20 trials daily with a fixed inter-trial interval of 20 s. The number of crossings between the chambers without a shock was recorded (inter-trial crossing). The automated shuttle box recorded this parameter during the whole experimental period of 20 trials (Hacioglu *et al.*, 2003).

Body weight gain

Body weight of male pups in each litter was recorded at birth and throughout the lactation period.

Statistical analysis

Statistical analysis was undertaken using MINITAB software (MINITAB, State College, PA, Version 13.1, 2002). Data from MDA, glutathione activity and differential counts were first tested for normality (using Anderson-Darling Normality test) and for variance homogeneity prior to any further analysis. Data were normally distributed and thus, one-way ANOVA was used to determine overall effects of treatments followed by individual comparison using Tukey's Pairwise comparison. The data obtained from grip strength, body weight gain and learning tests were compared within the experimental groups by analysis of variance (ANOVA) using MINITAB. Data were subsequently analyzed by Student's t-test (Yamane, 1973) for biochemical analyses and Mann-Whitney U tests (Sokal and Rohlfe, 1981) for behavioral analyses. Results are expressed as mean $\pm$ standard deviations (SD). Values of $P < 0.05$ were considered statistically significant.

RESULTS

To study the detrimental effects of sepsis in the mother on her pups, we used a mouse model in which injection of purified endotoxin is used to mimic Gram-negative infections. However, in general, rodents and primates are particularly insensitive to endotoxin when compared with humans. In fact, the lethal amount of disease-inducing endotoxin given to mice and rats ranges from a 1 to 10 mg/kg dose to produce a systemic inflammatory response. In contrast, in humans, an intravenous bolus injection of only 3 or 4 ng/kg of LPS produces fever, reduces hemodynamic functions, is a cardiosuppressant, and results in increased procoagulant activities (Kotb and Calandra, 2003). In the current study, we induce sepsis according to Starr *et al*. (2010) by a single dose of endotoxin (LPS) at a dose of 2.5 mg/kg of body weight and by intraperitoneal route.

Liver oxidative stress

Endotoxemia as a cellular stress resulted in oxidative stress, causing DNA and tissue damage. During gestation, oxidative stress affected embryonic development and organogenesis which resulted in the delay of different bioactivities of the pups after birth. Interestingly, we found an increase in oxidative stress which was represented by a significant increase of MDA in both LPS-treated mothers and their pups; this was seen in all tested tissues (Tables I, II). Glutathione levels were significantly suppressed in liver, brain and blood of pregnant females treated with LPS. Their pups also showed a significant decline of glutathione in comparison to control pups.

Hepatic and ileal histopathological investigations

After finding high oxidative stress in this study (MDA) and our previous study (NO) (Ebaid and Abdel-Salam, 2006), we investigated liver and ileal tissues. Liver sections from LPS-treated mice showed major tissue damage. The examination of liver sections of LPS-treated mothers showed that liver had lost its characteristic architecture compared with the control group, with abundant inflammatory cell infiltration around the central vein (Fig. 1). The cytoplasm of the hepatocytes in pups born to LPS-treated mothers was characterized by coarse, pink, darkly stained granules and vacuoles.

Damage in intestinal tissue, in particular enterocytes and villar structures, was detected histologically (Fig. 2A). Sections from pups born to endotoxin-treated mothers showed an increase in mitotic figures and apoptosis in crypts of Lieberkuhn (Fig. 2B) in addition to abnormally dilated submuscularis blood vessels (Fig. 2C).

Body weight of the newborns

Based on the histological findings of both liver and intestine, we monitored body weight during the first 21 days after birth. A significantly lower body weight increase was observed in the pups born to LPS-treated mothers comparing with control pups (Fig. 3).

Spleen histopathological changes and blood differential count

In a bacterial endotoxemia model, we have previously detected high antigen presenting cell-

Table I.- Oxidative stress (total glutathione and malondialdehyde, MDA) in liver homogenate, and total glutathione and total protein in blood of different groups of female mice.

			M	M-P	M-LPS	M-P-LPS
Liver	MDA nmol/g tissue	Mean	122.8	127.6	129.06	141.26*
		SD	11.8	2.3	6.3	11.8
		SE	5.2	1.02	2.8	5.3
	Glutathione	Mean	80.4	73.2	38.8*	30.2*
		SD	6.5	9.8	6.5	4.9
		SE	2.9	4.4	2.9	2.2
Blood	Glutathione %	Mean	89	68	62*	65*
		SD	11.6	13.4	7.08	8.09
		SE	4.9	5.2	3.5	4.2

SD, standard deviation; SE, relative error; * shows statistically significant differences at P < 0.05; M, female mice; M-P, Pregnant mice; M-LPS, LPS-treated female mice (non-pregnant); M-P-LPS, LPS-treated pregnant female mice.

Table II.- Oxidative stress (total glutathione and malondialdehyde, MDA) in liver homogenate, and total protein in blood of control pups (born to non-treated pregnant mice; M-P), and pups born to LPS-treated pregnant female mice (M-P-LPS).

		C-N	LPS-N
MDA (nmol/g tissue)	Mean	126.2	136.66*
	SD	2.2	7.6
	SE	1.02	3.4
Glutathione	Mean	60.2	46.4*
	SD	8.8	6.8
	SE	3.4	3.03

SD, standard deviation. SE, relative error; * shows statistically significant differences at P < 0.05.

activities with a high depletion of neutrophils in peripheral blood (Ebaid and Abdel-Salam, 2006). Spleen sections of pups born to LPS-treated mice showed highly distributed trabeculae. The red pulp was intensely congested with hemolyzed blood cells. The white pulp became much diffused with a large decrease in the lymphocytic population; many lymphocytes were observed with pyknotic nuclei. A large number of neutrophils were observed in the spleen sections. Numerous immature cells invaded the splenic pulp, with megakaryocytes of various stages occasionally infiltrating the spleen (Fig. 4).

In a bacteria endotoxemia model, we have previously detected high antigen presenting cell-activities with a high depletion of neutrophils in peripheral blood (Ebaid and Abdel-Salam, 2006). The differential leukocyte count revealed a significant increase (p □ 0.05) in the lymphocyte count of the LPS-treated mothers (81±10) and their pups (78±3.5) comparing to the control mothers (65±3.5) and pups (62±1.8) (Fig. 4D). On contrary, a significant low values (p □ 0.05) of neutrophils in the LPS-treated mothers (13±2) and their pups (18±3.5) comparing to the control mothers (27±3) and pups (30±6) was observed (Fig. 4E).

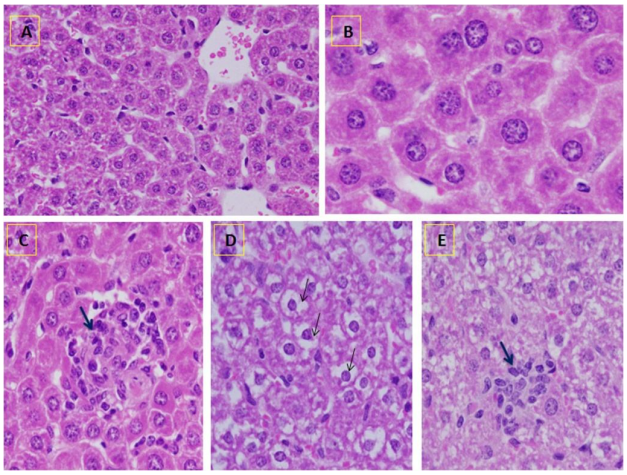

Fig. 1. A: A, representative control liver section showing the normal architecture (H&E, × 400). B, A representative liver section from an LPS-treated mother shows the degenerated nuclei (H&E, × 1000). C, The histological features of liver sections from LPS-treated mother showing increased inflammatory cell infiltration (H&E X400). D, A liver section from a newborn pup (LPS-treated mother) showing the increased vacuolation (arrows) and damage (H&E, × 400). E, A liver section from a newborn pup (LPS-treated mother) showing increased inflammatory cell infiltration (H&E, × 400).

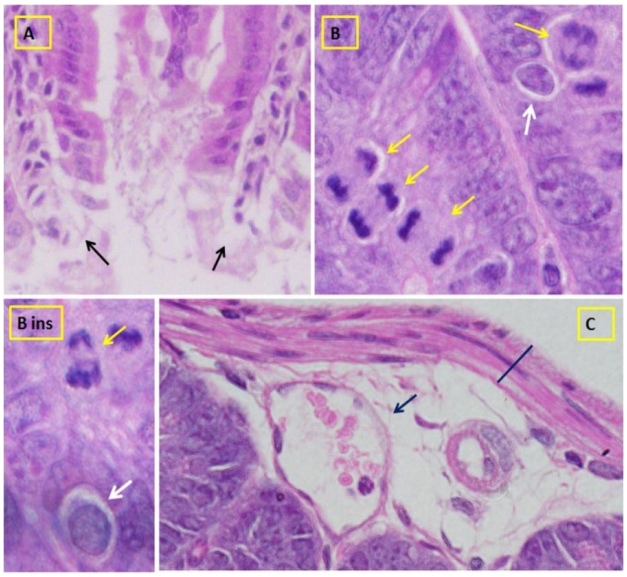

Fig. 2. A, Histological changes of the villi and enterocytes (black arrows) from pups born to endotoxin-treated mothers (H&E, × 400). B, A representative section from a pup born to an endotoxin-treated mother showing many mitotic figures (yellow arrows) and an apoptotic cell (white arrow) in crypts of Lieberkuhn (H&E, × 1000). B ins: A micrograph from the specimen in 2B showing apoptosis and mitosis (H&E, × 1000). C, A very thin longitudinal muscularis (line), and an abnormally dilated submuscularis blood vessel (arrow), in a representative section from a pup of a mother treated with LPS (H&E, × 1000).

Total protein of the blood and the muscle grip strength

The level of the blood total protein of the mothers treated with LPS was significantly decreased comparing to the control mothers. Unexpected significant increase of the total blood protein level was induced in the pups born to LPS-treated mothers (Fig. 5A).

The LPS-induced tissue damage for liver, intestine and spleen was histologically detected in pups. Based on these findings, we monitored changes in muscle activity (represented by grip strength), and cognitive ability (active avoidance test) in pups from the day of birth (PD1) until day 21 (PD21). Weakness was clearly observed in pups born to treated mothers, with significantly lower muscular grip strength recorded in the LPS group compared with pups born to control mothers (Fig.5B).

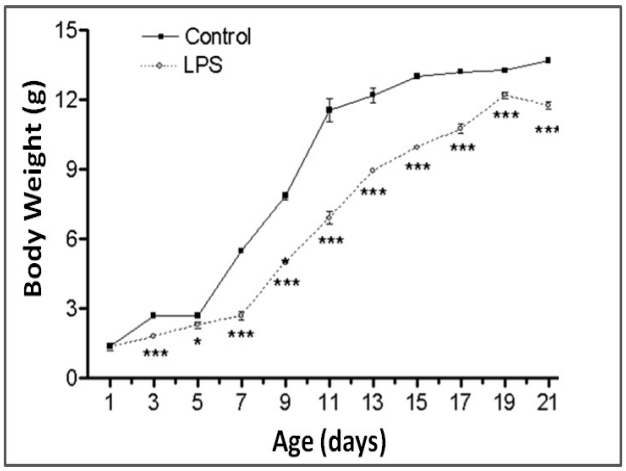

Fig. 3. The rate of body weight increase of pups from different treatments over time. This figure shows that a significantly lower body weight gain was recorded for pups from LPS-treated mothers compared with the control pups from the day of birth to 21 days after birth. Results are presented as mean and standard errors (SE); * and *** show statistically significant differences at $P < 0.05$ and at $P < 0.01$, respectively when treated compared with control animals.

Brain oxidative stress parameters and active avoidance learning of the newborns

An increased oxidative stress represented in a significant increase of MDA level in the brain tissues of LPS-treated mothers. Pups showed normal levels of MDA (Fig. 6). Glutathione levels was significantly suppressed in the brain of mothers treated with LPS. Their pups also showed a significant decline of the glutathione levels in comparison to control pups (Fig. 7).

As a direct results of the prenatal brain oxidative stress and the decrease of glutathione in pups, findings of the learning test in shuttle box showed that a significant impairment of active avoidance learning was clearly observed in pups born to LPS-treated mothers. Control pups learned to avoid the unconditioned stimulus by running into the other compartment during the unconditioned stimulus on almost all experimental days and recorded a high success rate of avoidance response (Fig. 8). The pups born to LPS-treated mothers did not learn to avoid the unconditioned stimulus and the number of crossings during the conditioned stimulus decreased over time during the experiment.

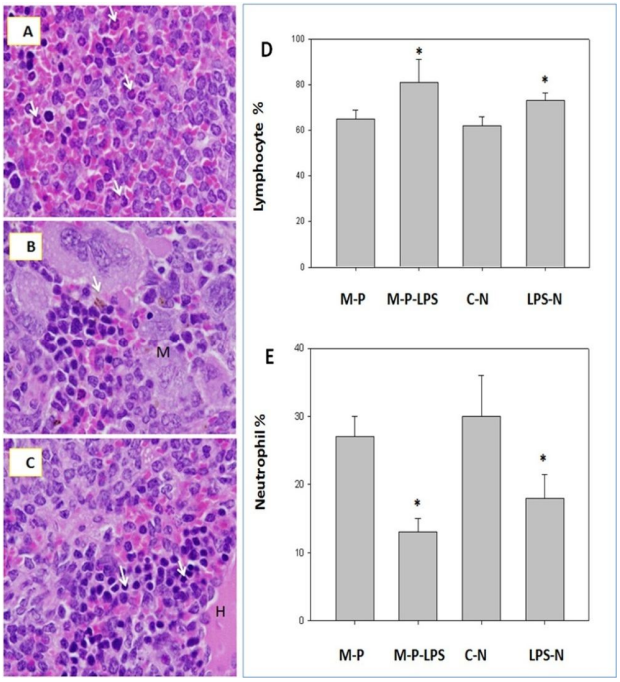

Fig. 4. A, spleen section from LPS-treated mothers showing infiltration of a large number of inflammatory cells, especially neutrophils (white arrows), into the red pulp (H&E, × 400). B, Spleen sections from pups born to LPS-treated mothers showing many immature stages of the megakaryocytes which had infiltrated the red pulp (M), and brown hemosiderin granules (white arrows) (H&E, × 400). C, Large numbers of pyknotic cells and hemolyzed blood are shown in spleen sections from the same pups (born to LPS-treated mothers) (H&E, × 400). The lymphocyte (D) and the neutrophil (C) percentages of control mothers (M-P), LPS-treated mothers (M-P-LPS), control newborn (C-N) and LPS newborn (LPS-N). Results are presented as a mean and a standard errors (SE); * shows statistically significant differences at $P < 0.05$ when treated compared with control animals.

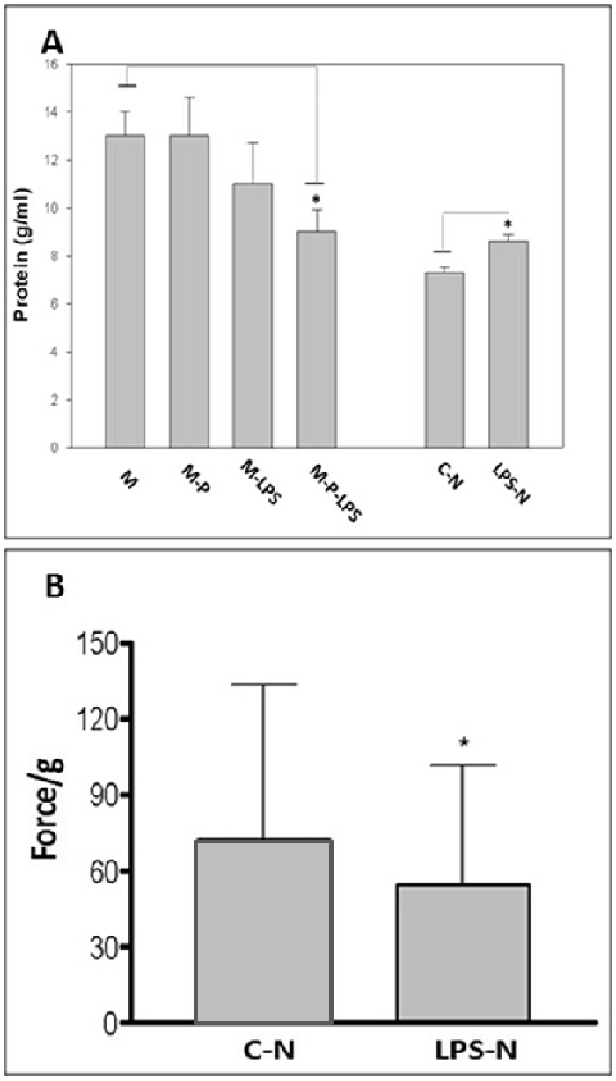

Fig. 5. A. The total protein level in blood of both mothers and their pups. Results are presented as mean ± SE. B, Evaluation of muscular strength using the Grip Strength apparatus. The mouse is placed over a base plate and when pulled by the tail, the mouse grasps at the bar, until the pulling force overcomes its grip strength. The Grip Strength Meter measures forelimb muscular grip strength in mice. Results are presented as mean ± SD. * shows statistically significant differences at $P < 0.05$.

DISCUSSION

Defense against pathogens is crucial, and involves antibacterial proteins, polysaccharides and other immune-related molecules. Although reactive oxidative species and free radicals are used by the body as cytotoxic materials against invading pathogens, they can also cause oxidative stress, leading to cellular damage. The current study aimed to obtain further evidence of oxidative stress as a pathogenic mechanism for endotoxemia and its effects on the offspring. Thus, we studied oxidative stress and histological changes in mothers and pups, and developmental determinants such as body weight gain, muscular grip strength and active

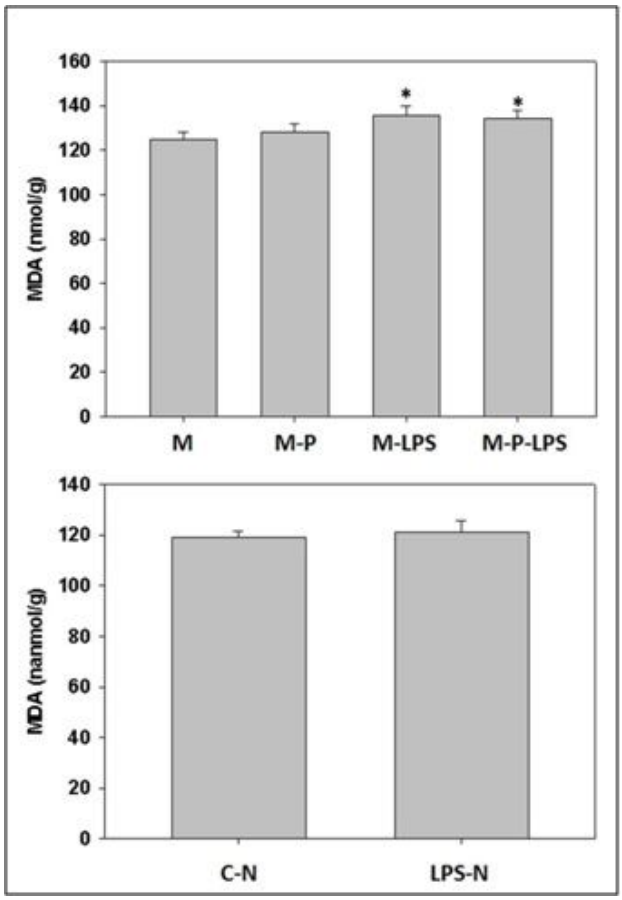

Fig. 6. Malondialdehyde (MDA) level in brain homogenates of different mother mice groups (A); M: control mice; M-P: Pregnant mice; M-LPS: LPS-treated mice (non-pregnant); M-P-LPS: LPS-treated pregnant female mice. B: MDA in brain homogenates of newborns from control mother (C-N) and LPS-treated mothers (LPS-N). * shows statistically significant differences at $P < 0.05$.

avoidance and learning in the pups, in response to endotoxemia.

Although different cellular stresses may have different initial responses, the final pathways are often similar. The mechanisms of cell injury are ATP depletion, disturbed calcium homeostasis, oxidative stress and increased membrane permeability. Bautista *et al*. (1990) observed an increase in O^{2-} generation by blood monocytes and PMN after administration of bacterial endotoxin. Seres *et al*. (2000) stated that the elimination of invading microorganisms depends on the generation of reactive oxygen species during the phagocytosis-associated respiratory burst. The oxidants created in this process therefore carry the potential to damage the phagocytes themselves as well as other cells at sites of inflammation (Henson and Rjb, 1987). Thus, bacterial agents activate neutrophils to secrete NO.

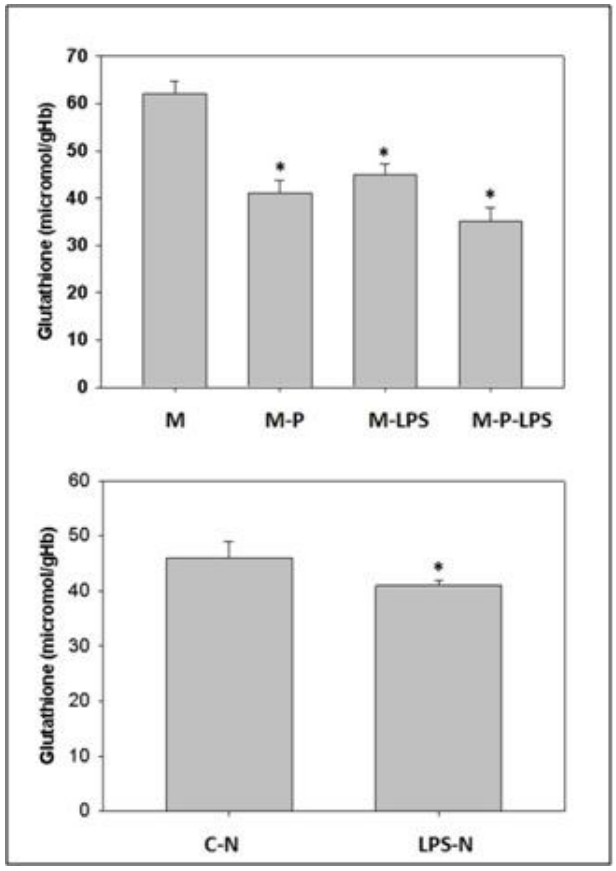

Fig. 7. Glutathione levels in brain homogenates of different female mice groups; M: control mice; M-P: Pregnant mice; M-LPS: LPS-treated mice (non-pregnant); M-P-LPS: LPS-treated pregnant female mice. Glutathione levels in brain homogenates of newborns from control mother (C-N) and LPS-treated mothers (LPS-N). * shows statistically significant differences at $P < 0.05$.

We have previously induced endotoxemia in adult mice using whole bacteria, and observed markedly increased NO synthesis, and decreased catalase, total peroxidase and superoxide dismutase activities (Ebaid and Abdel-Salam, 2006). ROS are radicals that can cause cellular damage such as lipid peroxidation, which disrupts membrane fluidity; degradation products can initiate cellular apoptosis

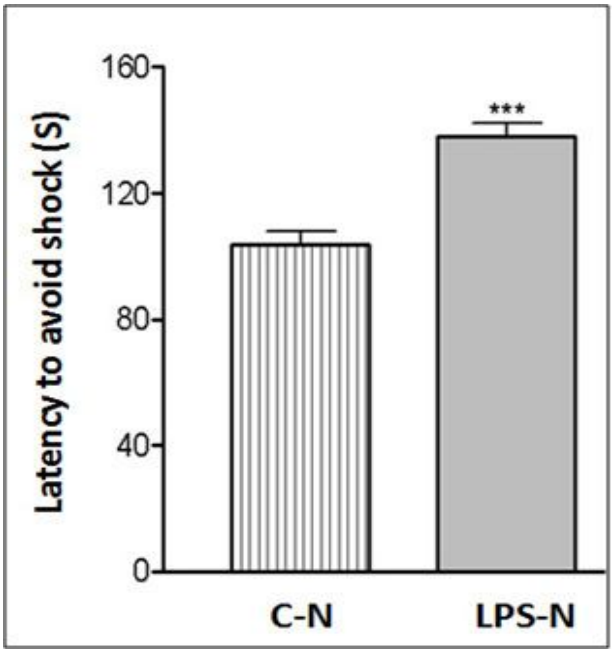

Fig. 8. The latency to avoid electric shock in a shuttlex box. Pups were tested to determine the time needed to learn how to avoid the electric stimulus by running into the other compartment during an unconditioned stimulus. Results are presented as a mean and a standard errors (SE); *** shows statistically significant differences at $P < 0.01$ when treated compared with control animals.

(Horton and Fairhurst, 1987; Halliwell and Gutteridge, 1999; Kannan and Jain 2000; Lopes *et al*., 2008; Golbidi and Laher, 2010). Thus, in the current study, oxidative stress is a potential cause of the tissue damage and impairment of various physiological and biochemical reactions in the body of both mothers and offspring. We found that MDA was significantly elevated in both LPS-treated mothers and their developing pups. Similar results were observed by Yazar *et al.*, (2010) who found that LPS increased MDA and organ damages markers. Functional studies in mammals have shown that glutathione prevents lipid peroxidation and protects biomembranes against oxidative stress (Ursini *et al.*, 1982). This is because glutathione is an antioxidant enzyme that can directly reduce peroxidized phospholipids and cholesterol within membranes (Ursini *et al.*, 1985; Thomas *et al.*, 1990). Based on these studies, the observed significant increase of lipid peroxidation after LPS treatment in the current study was associated with LPS-mediated suppression of glutathione production in the body of the treated mice and their pups. Thus, an oxidative imbalance (the formation of reactive oxygen species together with the depletion of GSH content) is the result of exposure to LPS, as has previously been shown (Scirocco *et al.*, 2010). In the present study, total levels of the anti-oxidant enzyme glutathione were significantly reduced in LPS-treated mothers and their pups. NO has direct effects on various enzymes that contain an iron-sulphur moiety in their catalytic center; such enzymes include ribonucleotide reductase (necessary for DNA synthesis) and several mitochondrial enzymes that are essential for cell survival (Karima *et al.*, 1999). Thus, it is more likely that the increased production of NO in the LPS-treated mice may be caused by the depletion of the antioxidant enzymes, which indeed showed decreased values.

Accordingly, the examination of liver sections showed major and obvious histopathological changes in the hepatic tissues. These changes were similar in both LPS-treated mothers and their pups. Similar results were obtained by Ebaid *et al.* (2007). In addition, there was extreme damage to the ileal mucosal barrier, in particular in pups born to LPS-treated mothers. As a direct result of the observed tissue damage, especially in the hepatic and ileal tissues, body weight gain was significantly reduced in the developing pups. Damage in the enterocytes and villar structures suppressed the absorption process, and metabolism in the born pups may therefore be greatly impaired. Our previous data (Ahmed and Ebaid, 2008, 2010) confirmed an increased influx of inflammatory and lymphocytic cells associated with apoptosis and hyper-mucus secretion, villar atrophy, necrosis and desquamation with infection. In addition, the reliance of young vertebrates on innate immunity and the associated inflammatory response often leads to reduced growth rates.

Cytokines also play a role in the pathogenesis of septic shock (Kotb and Calandra, 2003). In septic shock, the overproduction of pro-inflammatory cytokines contributes to manifestation of the systemic inflammatory response and development of organ failure (Kotb and Calandra, 2003). LPS

was found to increase levels of the inflammatory cytokines TNF-a, IL-6 and IL-8 (Chu *et al.,* 2010). The LPS-induced cytokine imbalance in turn depletes the lymphocytic population of B and T cells in splenic tissues, as was found in the current work. Spleen sections of pups born to LPS-treated mice showed major damage. A large number of neutrophils was observed in the spleen sections, which is likely to explain the neutrophil depletion which was recorded in the peripheral blood. Furthermore, Haraoka *et al.* (1999) found that the depletion of neutrophils may be due to the migration of antigen presenting cells across the epithelium from the blood pool to areas of tissue damage. Also they revealed that mediators alter blood flow and vascular permeability and increase the adherence of circulating phagocytes to the endothelial cells, thus promoting migration of leucocytes into tissue to clear infection.

Young vertebrates depend on innate immunity and the associated inflammatory response that often leads to changes in behavior after antigenic challenge. Experiments investigating locomotor activity indicate that LPS significantly reduces these activities in pups when compared with control animals. Postnatal development is a crucial indicator of the stress associated with prenatal LPS exposure. Because LPS-associated oxidative stress continues during embryogenesis and development, a direct change in behavioral elements may be observed. The learning ability of animals was therefore addressed to determine effects of LPS exposure. Results of the learning tests (specifically the shuttle box) showed that LPS impaired the avoidance conditioning of pups born to treated mothers. Similar results were obtained by Ajarem *et al.* (2011) and Al basher *et al.* (2011). Neurons and muscle cells are extremely sensitive to cellular stress, which usually occurs as a consequence of ischemic injury. Feng *et al.* (2010) found histological evidence of brain injury, and thus concluded that prenatal injection of endotoxin impairs circulation in newborn animals, and this may place the newborn brain at prolonged risk of cerebral endothelium and cerebral blood flow dysregulation and injury as a legacy of endotoxin exposure. This may explain our results showing impairment of learning (active avoidance in the shuttle box).

In conclusion, evidence of detrimental effects of LPS on biochemical, histological and developmental outcomes was found in this study, and thus, prenatal injection of endotoxin impairs organ structure and function in the newborn. Endotoxin-induced elevation of oxidative stress and suppression of glutathione may place the embryos and the developing pups at prolonged risk of tissue damage by free radicals. The imbalance of the oxidative stress response may lead to major subsequent damage (both structural and functional) in a variety of tissues in mothers and pups. Taken together, these results showed a positive correlation between oxidative stress-imbalance resulting from endotoxemia, and pathological changes transferred to the pups born to LPS-treated mothers. The knowledge of these interactions may provide novel information on the risks associated with prenatal exposure to bacterial endotoxin-associated pathogenesis, and further clues for the development of new treatment or prevention strategies.

ACKNOWLEDGEMENT

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

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(Received 3 November 2011, revised 17 December 2011)