

Evaluation of immunomodulatory effects of lamotrigine in BALB/c mice

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Accepted August 8, 2017
Published online September 13, 2017

Modulation of the immune system has recently been shown to be involved in the pharmacological effects of old anti-epileptic drugs and in the pathogenesis of epilepsy. Therefore, the most recent guidelines for immunotoxicological evaluation of drugs were consulted to investigate the immunomodulatory effects of lamotrigine, a newer anti-epileptic drug, in BALB/c mice. These included the *in vivo* effects of lamotrigine on delayed-type hypersensitivity (DTH) response to sheep red blood cell (SRBC) antigens, hemagglutination titer assays and hematological changes. *In vitro* effects of lamotrigine on ConA-induced splenocyte proliferation and cytokine secretion were assessed. The results showed that lamotrigine treatment significantly increased the DTH response to SRBC in the mouse model of this study. This was accompanied by a significant increase in relative monocyte and neutrophil counts and in spleen cellularity. Lamotrigine significantly inhibited ConA-induced splenocyte proliferation *in vitro* and it significantly inhibited IL-2 and TNF- α secretion in ConA-stimulated splenocytes. In conclusion, the results demonstrated significant immunomodulatory effects of lamotrigine in BALB/c mice. These data could expand the understanding of lamotrigine-induced adverse reactions and its role in modulating the immune system in epilepsy.

Keywords: lamotrigine, epilepsy, cytokine, hypersensitivity, immunomodulatory effect, immunotoxicity

Immune system modulation by drugs can result in adverse effects or additional therapeutic effects. This alteration might be unintended immunosuppression or immunoenhancement. Therefore, the US Food and Drug Administration (FDA) and the International Conference on Harmonization (ICH) guidelines for immunotoxicity evaluations of human pharmaceuticals recommended immunotoxicity evaluation for drugs in animals to delin-

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evaluate such potential effects on immune responses (1–3). Antiepileptic drugs are a class of drugs with documented immunomodulatory effects, apart from their intended pharmacological effects involved in the treatment of epilepsy. On the one hand, these immunomodulatory effects have been reflected in a range of immunological/allergic adverse reactions from mild cases of rashes and urticaria to the potentially life threatening reactions with eosinophilia and systemic symptoms (DRESS), toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS) (4). On the other hand, more importantly, there is an increasing body of evidence for the potential involvement of the immunomodulatory characteristics of antiepileptic drugs in their therapeutic benefits in the treatment of epilepsy. It has recently been shown that modulation of the immune system and inflammation are implicated in the pathogenesis of epilepsy (5). This partially involves alteration in cytokine secretion, in particular, increased pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α production (6–8). IL-2, in addition, was found to promote seizure generation in various murine models of epilepsy (9). Several reports have shown that antiepileptic drugs cause alteration in the cellular and humoral immune responses, both *in vitro* and *in vivo*. In this context, most of the relevant data are focused on only a few classical antiepileptic agents, such as carbamazepine, phenytoin and valproate (10–13). However, studies performed on newer antiepileptic drugs, such as lamotrigine, are rare and focused on *in vitro* testing (11–13).

Lamotrigine is a phenyltriazine anticonvulsant that inhibits neuronal voltage-sensitive sodium channels. It is indicated for the management of certain types of epilepsy and for bipolar disorder (14). Evaluation of the immunomodulatory effects of lamotrigine has become pivotal due to its allergic and hypersensitivity adverse effects. This is also due to its effects on the immune system, as described before, in patients with epilepsy where these effects could be of therapeutic benefit (14–16). Considering its adverse effects, a black box warning was introduced into the monograph of this drug in 2010 owing to the serious hypersensitivity adverse effects that have occurred with lamotrigine treatment such as SJS, DRESS, and drug-induced hypersensitivity syndrome (14, 15). With regard to its immunomodulatory effects in epilepsy, low levels of immunoglobulins have been observed in the sera of epileptic patients treated with lamotrigine (16). *In vitro*, lamotrigine has been shown to inhibit IL-2, IL-1 β and TNF- α secretion, but not IL-6 secretion, in stimulated-human whole blood (12), while it had no effect on murine splenocyte proliferation *in vitro* (11).

Despite the available data on immunomodulatory effects of lamotrigine, no *in vivo* murine model for the evaluation of the immunomodulatory effects of lamotrigine, based on the current pertinent FDA and ICH guidelines (1, 2), is available. Therefore, the aim of this work was to characterize the effect of lamotrigine on cellular and humoral immune responses in a 21-day *in vivo* study in BALB/c mice. This included the *in vivo* evaluation of cellular responses (delayed-type hypersensitivity, DTH) and humoral responses (hemagglutination titer assay) to a foreign antigen. Other *in vivo* testing involved the evaluation of changes in animal mass, lymphoid organ mass (thymus and spleen), spleen cellularity, and total and differential white blood cell (WBC) count. To the best of our knowledge, no previous reports have been conducted to assess the effects of lamotrigine on mitogen (concanavalin A [ConA])-induced cytokine secretion in isolated splenocytes *in vitro*. Hence, this effect of lamotrigine on immune responses *in vitro*, along with its effect on splenocyte proliferation, was investigated in this study.

EXPERIMENTAL

Animals

Female BALB/c mice (6 to 8 weeks old, 18–22 g) were obtained from the Applied Science University's animal house, Amman, Jordan. Animals were housed in plastic cages with free access to laboratory diet and water. Animals were acclimatized for 1 week in the laboratory prior to the experiment. The animals' room was maintained at ambient temperature of 20–25 °C, relative humidity of 40–70 % and a 12 h light/dark cycle. Ethical approval of the study protocol was obtained from the Scientific Committee, School of Pharmacy, The University of Jordan (Amman, Jordan).

Reagents

Lamotrigine powder was purchased from Sana Pharma (Jordan). SRBC suspension, RPMI-1640 medium, penicillin-streptomycin solution, fetal bovine serum (FBS), ConA (from *Canavalia ensiformis*) and thiazoyl blue tetrazolium bromide (MTT) were all purchased from Sigma-Aldrich Chemical Co. (USA).

Lamotrigine preparation

For *in vivo* experiments, a stock solution of lamotrigine in polyethylene glycol (PEG) (12.5 mg mL⁻¹) was freshly prepared daily while for *in vitro* experiments, a stock solution of 12 mg mL⁻¹ of lamotrigine was prepared in dimethyl sulfoxide (DMSO) and stored at -20 °C.

Dosage and treatment protocol for in vivo experiments

Mice were randomly divided into five treatment groups of 7 mice each. Mice in the control group received 0.2 mL of 20 % PEG in a phosphate buffer saline (PBS) solution intraperitoneally (*i.p.*). The other groups received 0.2 mL of 1.5, 6, 12, or 24 mg kg⁻¹ bm of lamotrigine *i.p.* daily for 21 days, corresponding to 0.25-, 1-, 2-, or 4-times the human therapeutic dose, resp. Four hours after administration of the last dose, mice were anesthetized with diethyl ether inhalation before retro-orbital blood collection for serum preparation and WBC count determination. Mice were then sacrificed by cervical dislocation for thymus gland and spleen collection.

Organs and body mass

The mass of each animal was recorded on day 1 and on day 21 (terminal body mass) just before dosing. On day 21, four hours after injection, animals were sacrificed and thymus glands and spleens were extracted. Organs were weighed after the removal of connective and adipose tissues. Relative organ masses were calculated as the ratio of organ mass (mg) / terminal body mass (g).

Total and differential WBC count

On day 21, before animals were sacrificed, blood was collected from the retro-orbital plexus using heparinized capillary tubes. Blood from each mouse was collected into an

EDTA tube. The total WBC count was then determined manually under a light microscope. For the differential WBC count, blood films were prepared and stained with polychrome methylene blue-eosin stain (Diamond, Jordan) and then cells were counted under the light microscope in a blinded fashion. Three blood films were prepared and examined for each mouse.

Single splenocyte cell suspension (SSCS)

For the assessment of spleen cellularity, after treatment with lamotrigine *in vivo*, the spleen from each mouse was individually collected in a sterile Petri dish containing 10 mL of RPMI-1640 medium supplemented with 10 % FBS. Spleens were minced between the frosted edges of two microscopic slides and the cells were then collected and centrifuged at 1200 rpm for 7 min at 4 °C in a refrigerated centrifuge (Sigma, USA). The red blood cells (RBC) within the pellets were then lysed using RBC lysis buffer (0.83 % NH₄Cl in 100 mmol L⁻¹ Tris buffer, pH 7.4) and kept at room temperature (RT) for 3 min. Lysed RBC were then removed by washing the cells three times with 10 mL of RPMI-10 % FBS at 1200 rpm for 7 min at 4 °C. The remaining splenocytes were then suspended in 1 mL of RPMI-10 % FBS and the viable splenocyte count was determined using the trypan blue dye exclusion method (17).

For *in vitro* experiments, SSCS was prepared as described above; however, aseptic surgical excisions of spleens were performed and sterile tissue culture techniques were employed.

Serum antibody titer: hemagglutination log₂ titer assay

Seven days before the end of the *in vivo* experiment (on day 14), mice were intraperitoneally injected with 5×10^8 SRBC in 0.2 mL of PBS. On day 21, four hours after lamotrigine dosing, blood was collected from the retro-orbital plexus into silica gel-containing plain tubes (Minicollect®, Greiner, Germany). Blood samples were left to clot at RT for 30 min and were then centrifuged at 3000 rpm for 10 min at 4 °C before serum collection. In a round-bottomed 96-well microtiter plate, serial dilution of the sera in PBS (from 1:2 to 1:2048) was conducted. An equal volume of 1 % (V/V) SRBC suspension in PBS was added to 25 µL of diluted serum, mixed, covered and incubated at 37 °C for 1 h. The plates were then visually observed for hemagglutination. Hemagglutination titer was defined as the highest dilution of the serum (log₂ for the highest dilution) after which the hemagglutination lattice structure could not be observed. The method was modified from Fararjeh *et al.* (17).

DTH response to SRBC

On day 14 of the experiment, the mice were injected subcutaneously with 1×10^9 SRBC per 50 µL of PBS. On day 20, the mice were re-challenged with a booster dose of SRBC by injecting 1×10^8 SRBC per 10 µL PBS into their right hind foot pad. The left hind foot pad of each mouse was injected with an equal volume of PBS as trauma control. Twenty-four hours later (day 21), footpads were collected after sacrificing. Right and left footpad volumes were then measured using a digital plethysmometer LE 7500 (Panlab Harvard Apparatus, USA) and the differences between their volumes were calculated (17).

Cell cultures

SSCS was prepared aseptically as described above. Cultures of splenocytes (2×10^6 cells mL^{-1}) were suspended in complete RPMI-1640 medium containing 10 % FBS, 2 mmol L^{-1} L-glutamine, 100 U mL^{-1} penicillin, 100 $\mu\text{g mL}^{-1}$ streptomycin, 0.05 mmol L^{-1} 2-mercaptoethanol, 1 mmol L^{-1} sodium pyruvate and $1 \times$ non-essential amino acids (Euroclone, Italy). Cultures were maintained at 5 % CO_2 and 90–95 % humidity at 37 °C. The concentration range of lamotrigine (3–24 $\mu\text{g mL}^{-1}$) was chosen on the basis of therapeutic concentrations used in humans and previous studies in mice (11, 12, 18). In addition, a concentration higher than the therapeutic range was also tested (48 $\mu\text{g mL}^{-1}$).

In vitro cellular proliferation assay

Cultures of splenocytes were prepared in flat-bottomed 96-well microplates (4×10^5 cells per 200 μL per well). Splenocytes were either treated with lamotrigine at final concentrations of 3, 6, 12, 24 or 48 $\mu\text{g mL}^{-1}$ or were left untreated (solvent control) and incubated for 1 h. Cultures were then co-treated with 1 $\mu\text{g mL}^{-1}$ of ConA for 24, 48 or 72 h. Two hours prior to the end of each incubation interval, an MTT assay was performed as previously described (19). Absorbance was then measured at 570 nm using a microplate reader (Biotek, USA). The cell viability percent of the control (viability %) was calculated.

Cytokine secretion by splenocytes in vitro

Cultures of splenocytes were prepared in 24-well plates (2×10^6 cells per mL per well). Splenocytes were either left untreated (solvent control) or were treated with lamotrigine at final concentrations of 3, 6, 12 or 24 $\mu\text{g mL}^{-1}$ and incubated for 1 h. Cultures were then co-treated with 1 $\mu\text{g mL}^{-1}$ of ConA for 24 h. Culture supernatants were collected and used to assess IL-2, IL-6 and TNF- α secretion using a Mouse TNF alpha ELISA Ready-SET-Go!TM Kit (eBiosciences, USA) as per manufacturer's protocols. The final concentration of cytokines is presented as pg mL^{-1} of culture supernatant.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Dunnett's post-hoc analysis testing using Prism 5 software (GraphPad, USA). Data are presented as mean \pm standard error of the mean (SEM).

RESULTS AND DISCUSSION

Effects of lamotrigine on immune system parameters in vivo

Assessment of the *in vivo* effects of drugs on animal body and organ mass, hematological parameters and on cellular and humoral immune responses is recommended by the FDA and ICH guidelines (1–3). Therefore, the effect of lamotrigine on these parameters was investigated in this study.

Table I. Effect of lamotrigine on terminal body mass and relative organ masses of female BALB/c mice

| Lamotrigine (mg kg ⁻¹ bm) | Initial body mass (g) ^a | Terminal body mass (g) ^a | Relative spleen mass (%) ^a | Relative thymus mass (%) ^a |
|---|---------------------------------------|--|--|--|
| Control (20 % PEG/PBS) | 19.00 ± 0.98 | 24.50 ± 0.82 | 1.23 ± 0.06 | 0.37 ± 0.11 |
| 1.5 | 19.86 ± 1.24 | 24.60 ± 0.51 | 1.47 ± 0.12 | 0.44 ± 0.07 |
| 6 | 20.71 ± 1.37 | 26.17 ± 0.70 | 1.36 ± 0.13 | 0.37 ± 0.03 |
| 12 | 20.43 ± 1.27 | 26.14 ± 0.83 | 1.27 ± 0.12 | 0.34 ± 0.05 |
| 24 | 20.43 ± 1.46 | 26.33 ± 1.09 | 1.36 ± 0.14 | 0.33 ± 0.04 |

^a Mean ± SEM (*n* = 7).

Body and organ mass. – As shown in Table I, lamotrigine treatment in four doses over 21 days did not induce any significant changes in animal terminal body mass, relative thymus mass or spleen mass compared to the corresponding masses in the control group. The observed increase in terminal body mass in comparison with the initial body mass indicated a normal growth rate for the animals at the age of 6–8 weeks. This is in line with a previous study in humans, which showed that a maintenance treatment with lamotrigine for one year induced no significant change in the subjects' body masses compared to placebo (20).

WBC count and differential. – Treatment of mice with lamotrigine for 21 days was associated with a significant *in vivo* increase in total circulating WBC count at 6 and 12 mg kg⁻¹ bm doses only compared to the untreated control group (Fig. 1a). Differential WBC staining showed that the relative circulating neutrophil count had increased significantly at a 24 mg kg⁻¹ bm dose only, while the relative circulating monocyte count had increased at both 12 and 24 mg kg⁻¹ bm of lamotrigine (Figs. 1b, c). However, lamotrigine administration had no effect on the relative lymphocyte count (Fig. 1d). Eosinophil counts were very low in all samples and therefore data are not shown.

Spleen cellularity. – In addition to assessment of the lamotrigine effect on spleen mass, its effect on spleen cellularity was also assessed. While data (Table I) showed that the net spleen mass was not significantly changed by lamotrigine treatment, lamotrigine, however, induced a significant increase in spleen cellularity over the whole dose range (1.5–24 mg kg⁻¹ bm) over 21 days compared to the control (Fig. 2). As a lymphoid organ, the spleen contains a large reserve of lymphocytes and monocytes (21).

Cellular and humoral immune responses in vivo. – The induction of circulating WBC and splenocyte production after treatment with lamotrigine (as described above) suggests that the immune function and responses could be altered with lamotrigine treatment. To test this, the effects of lamotrigine on the DTH response to an exogenous antigen (SRBC) and on antibody production to this antigen (serum antibody titer) were investigated.

An assay of DTH response to antigens was performed to assess the effect of lamotrigine on the cell-mediated immunity. The DTH response is a T-cell and macrophage depen-

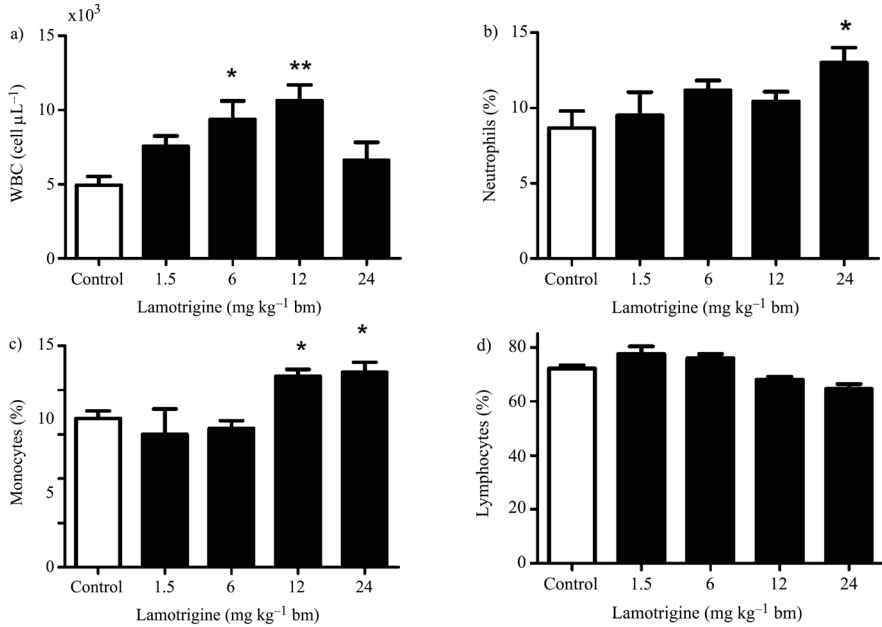


Fig. 1. Effects of lamotrigine on circulating WBC count: a) total, b-d) differential. Female BALB/c mice were daily injected with lamotrigine *i.p.* at 1.5, 6, 12 or 24 mg kg⁻¹ bm or left untreated (20 % PEG/PBS, control) for 21 days before retro-orbital blood collection and manual counting of the indicated cells under a light microscope (mean \pm SEM, $n = 7$). Significant difference *vs.* control: * $p < 0.05$, ** $p < 0.01$).

dent immune response to antigens (22). The antigen used in this study to induce the DTH response in mice was SRBC. SRBC was injected subcutaneously to lamotrigine-treated and untreated mice, followed by a re-challenged dose in the paw, which resulted in enhanced

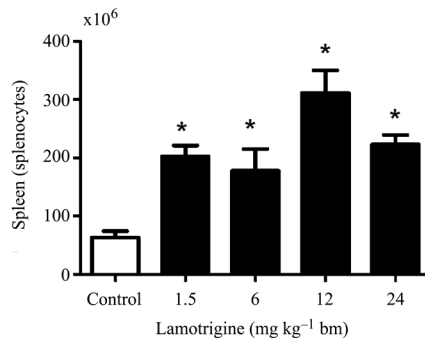


Fig. 2. Spleen cellularity assessment in female BALB/c mice after daily *i.p.* injections of lamotrigine (1.5, 6, 12 or 24 mg kg⁻¹ bm) or injection of 20 % PEG/PBS (control) for 21 days before spleen extraction. Manual counting of splenocytes in SSCS was performed under a light microscope (mean \pm SEM, $n = 7$). Significant difference *vs.* control: * $p < 0.05$.

recruitment of cellular infiltrate to the paw (as described in the methods). The results of this study showed that the DTH response to SRBC was significantly induced by lamotrigine at the four doses tested compared to the control group (Fig. 3a). This was expressed as the difference in paw volume between SRBC-injected and PBS-injected paws. Based on ICH guidelines, enhancement of the immune response can exaggerate hypersensitivity reactions (2). In this context, previous case reports described DTH reaction after treatment with lamotrigine as cases of lamotrigine-induced DRESS (15), where DRESS was defined as a type of cell-mediated immune stimulation (DTH reaction) (23). Therefore, the results of the current study support the notion that lamotrigine can enhance cellular immune responses and can exaggerate DTH reactions.

The effect of lamotrigine on humoral immunity and antibody production in response to antigenic stimulation was assessed using the hemagglutination titer assay (22). Lamotrigine-treated and untreated mice were injected with SRBC. Anti-SRBC antibody titers were determined in the sera (as described in the methods). The results of this study showed that lamotrigine, in the four doses tested, had no effect on the concentration of the anti-SRBC antibody (expressed as antibody titer) and thus had no effect on B-cell-mediated antibody production to SRBC (Fig. 3b).

Immune function responses in vitro. – The mitogen-induced splenocyte stimulation assay was used to test the *in vitro* immune functions of lamotrigine. This *in vitro* assay is considered similar to the *in vivo* DTH assay regarding the assessment of immune cell response to antigens (22). Therefore, the effect of lamotrigine on the inflammatory responses of splenocytes treated with a mitogen (ConA) *in vitro* was tested. This included splenocyte proliferation response using the MTT assay and cytokine secretion response using ELISA.

ConA-induced splenocyte proliferation in vitro. – ConA-induced splenocyte proliferation was significantly inhibited following the treatment with lamotrigine in the whole concentration range 12–48 $\mu\text{g mL}^{-1}$ after 48 and 72 h of incubation but not after 24 h (Fig. 4). However, Basta-Kaim *et al.* (11) showed that lamotrigine had no effect on ConA-induced splenocyte proliferation *in vitro* after 72 h of incubation. The difference between these results and the results of the current study could be ascribed to the difference in experimental

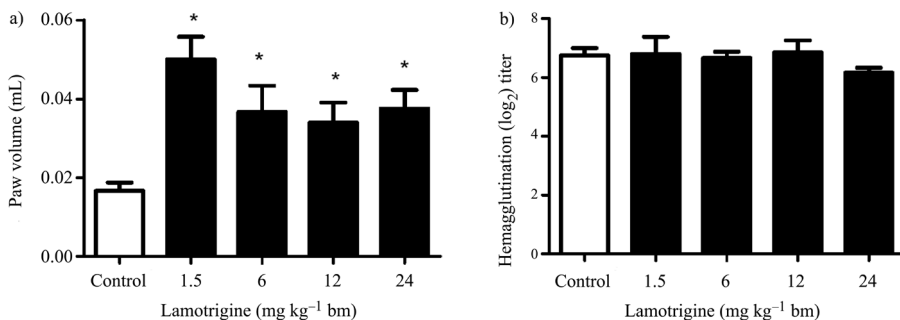


Fig. 3. Lamotrigine-enhanced murine delayed-hypersensitivity response to SRBC in female BALB/c mice daily injected with lamotrigine *i.p.* at 1.5, 6, 12 or 24 mg kg⁻¹ bm or left untreated (20 % PEG/PBS, control) for 21 days. a) DTH assay, b) hemagglutination titer assay (log₂ of the highest dilution was used as the antibody titer (mean \pm SEM, $n = 7$). Significant difference *vs.* control: * $p < 0.05$.

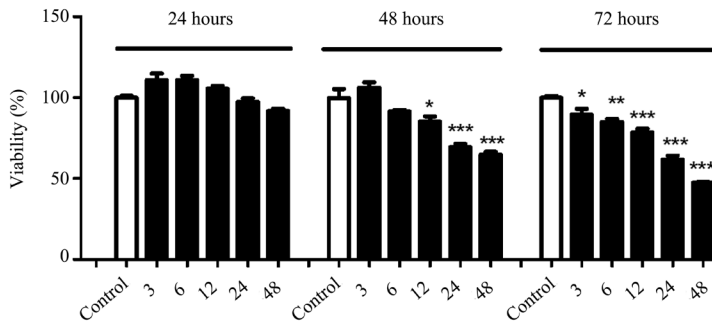


Fig. 4. Lamotrigine inhibited the proliferation of ConA-stimulated splenocytes *in vitro*. Cultures of isolated splenocytes were treated with lamotrigine at concentrations of 3, 6, 12, 24 and 48 $\mu\text{g mL}^{-1}$, or were left untreated (control) for 1 h before being stimulated with ConA ($1 \mu\text{g mL}^{-1}$) for 24, 48 or 72 h. Cellular proliferation was measured using MTT assay (mean \pm SEM, $n = 4$, each experiment in triplicate). Significant difference vs. control: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

conditions, such as isolating splenocytes from male C57BL/6 mice of about 3 months of age, while in this study female BALB/c mice of 1.5–2 months of age were used. In this regard, strain- and sex-associated differences in the regulation of immune responses in mice have been reported previously (24, 25). In addition, in contrast to the results of this study, Naisbitt *et al.* (26) demonstrated that lamotrigine enhanced the proliferation of peripheral blood mononuclear cells (PBMC) isolated *in vitro* from patients with lamotrigine-hypersensitivity. This discrepancy could be attributed to the use of human white blood cells, isolated from patients with epilepsy in the report of Naisbitt *et al.*, whereas the current study demonstrated effects of lamotrigine on murine splenocytes isolated from mice without epilepsy. In this regard, several immunological changes have been previously reported to be involved in the pathogenesis of epilepsy that cannot be separated from those of antiepileptic drugs (27).

Cytokine secretion in ConA-stimulated splenocytes in vitro. – The effect of lamotrigine on cytokine secretion by ConA-stimulated splenocytes *in vitro* was studied after 24 h of incubation as lamotrigine had no effect on cell viability at this time point. Lamotrigine treatment of ConA-treated splenocytes resulted in a dose-dependent inhibition of TNF- α and IL-2 secretion, in particular, following the treatment with 12 and 24 $\mu\text{g mL}^{-1}$. However, lamotrigine treatment for 24 h had no effect on IL-6 secretion in ConA-treated splenocytes *in vitro* in the concentration range tested (Figs. 5a–c). These results explain the reduction in ConA-induced splenocyte proliferation presented in this study. ConA has been long used to activate and induce the proliferation of T-lymphocyte population within murine splenocytes (28). T-lymphocyte population accounts for about 40–50 % of immune cells within the murine spleen (29). Once activated, T-lymphocytes secrete IL-2, which is considered to be the main growth factor that stimulates T-lymphocyte proliferation (30). Therefore, the reduction in splenocyte proliferation by lamotrigine could be attributed to the inhibitory effect of lamotrigine on ConA-induced IL-2 production. As regards TNF-alpha, it is mainly produced by monocytes; in addition, it is secreted by activated T-lymphocytes (30). Therefore, the reduction in TNF- α production could be partially attributed also to the in-

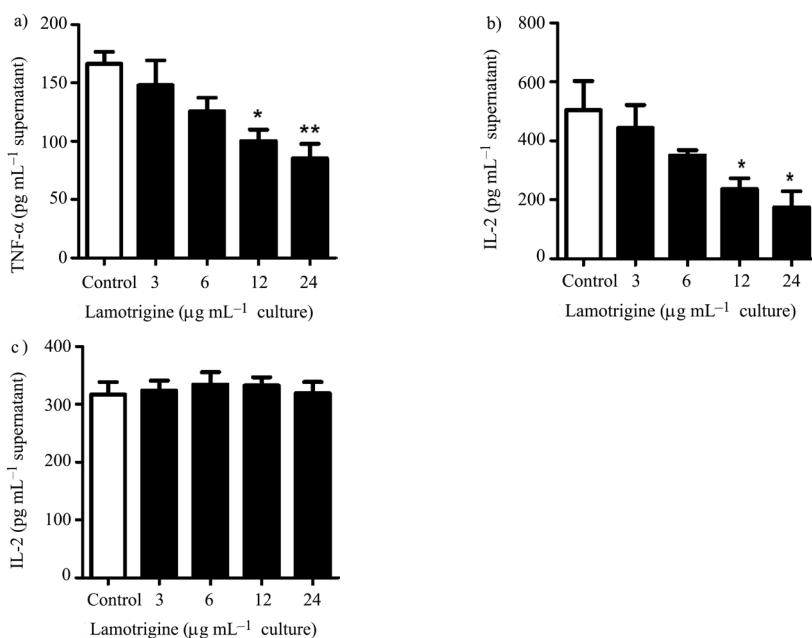


Fig. 5. Effects of lamotrigine on TNF- α , IL-2 and IL-6 secretion in ConA-stimulated splenocytes *in vitro*. Cultures of isolated splenocytes were treated with lamotrigine at concentrations, of 3, 6, 12, 24 and 48 $\mu\text{g mL}^{-1}$ or were left untreated (control) for 1 h before being stimulated with ConA ($1 \mu\text{g mL}^{-1}$) for 24 h. Culture supernatants were used to assess: a) TNF- α levels, b) IL-2 levels, c) IL-6 levels, using ELISA (mean \pm SEM, $n = 4$). Significant differences *vs.* control: * $p < 0.05$, ** $p < 0.01$.

hibitory effect of lamotrigine on ConA-induced T-cell activation. IL-6 is a T-helper 2 cytokine mainly produced by T-cells and macrophages (30). The results of this study showed that lamotrigine had no effect on ConA-induced IL-6 production by murine splenocytes. This might be attributed to a possible difference in the effect of lamotrigine on the production of cytokines by the different subsets of T-cells, where IL-2 is a T-helper 1 cytokine while IL-6 is a T-helper 2 cytokine (30).

Compared to previous reports, similar effects of lamotrigine on the three cytokines have been found after stimulation of whole blood obtained from healthy donors, with toxic shock syndrome toxin-1 (TSST-1) (12). However, another study showed that lamotrigine inhibited IL-2 and IL-6 secretion but not TNF- α secretion in human whole blood after stimulation with a combination of anti-CD3 and anti-CD40 antibodies (12). Therefore, the use of different stimulants could result in different immunomodulatory effects of lamotrigine. Regarding epilepsy, inhibition of IL-2 and TNF- α production might be beneficial in the management of epilepsy as these cytokines were found to be involved in the pathogenesis of epilepsy (6, 7).

In vivo results of the current study demonstrated enhancement of several parameters within the immune system after lamotrigine treatment. The *in vitro* results, though, demonstrated that lamotrigine inhibited ConA-induced splenocyte proliferation and IL-2 and

TNF- α secretion. In comparison with other antiepileptic drugs, the majority of classical antiepileptic drugs exert immunosuppressive properties; however, many of them can still enhance the immune system (4). Clinical studies revealed that several immunological parameters and the levels of several immunoglobulins were depressed in patients treated with carbamazepine or phenytoin (10). However, carbamazepine and phenytoin resulted in elevated blood levels of IL-2 and IL-1 in patients with epilepsy, respectively, while valproic acid resulted in increased IL-1, IL-6 and IL-5 blood levels (4, 8). In contrast to *in vivo* studies, carbamazepine, phenytoin and valproic acid inhibited IL-2, IL-4, IL-6 and TNF- α secretion *in vitro* (4, 11–13). Thus, reports on the effects of antiepileptic drugs on the immune system are commonly inconsistent or conflicting because of different experimental conditions in the different studies and because the effects of the drugs cannot be separated from those of seizures (27). In addition, different types of epilepsy are characterized by different patterns of immunological alteration (31). Consequently, the results of this study emphasize the immunomodulatory effects of antiepileptic drugs, particularly lamotrigine, which could be enhancement or inhibition of the immune system depending on the experimental conditions. This explains the fact that lamotrigine might result in hypersensitivity reactions as a case of immune system stimulation while it can still exert beneficial effects in modulating the inflammatory state in patients with epilepsy.

CONCLUSIONS

Findings of the present study demonstrated that lamotrigine had significant immunomodulatory properties in mice, both *in vivo* and *in vitro*. This was shown in enhancement of relative monocyte and neutrophil counts, increased spleen cellularity, and enhanced cellular adaptive immune response *in vivo*, as shown in the enhanced DTH response to SRBC. Lamotrigine, in addition, resulted in a significant suppression of ConA-induced splenocyte proliferation and IL-2 and TNF- α secretion *in vitro*. These results provide insights into possible effects implicated in lamotrigine-induced immunological adverse effects and possibly further clarify its role in modulating the immune system in epilepsy. Therefore, the development of immunological adverse effects, and seizure control, should be carefully monitored during lamotrigine treatment. Further studies, however, are necessary to investigate the detailed mechanisms behind the immunomodulatory effects of lamotrigine and to investigate these effects during chronic exposure protocols.

Acknowledgements. – This work was supported by the Deanship of Academic Research, the University of Jordan.

REFERENCES

1. Food and Drug Administration, Center for Drug Evaluation and Research, U.S. Department of Health and Human Services, *Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs*, October 2002; <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm079239.pdf>; last access date July 9, 2017.
2. International Conference on Harmonisation, *Harmonised Tripartite Guideline: Immunotoxicity Studies for Human Pharmaceuticals S8*, May 2005; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002851.pdf; last access date July 9, 2017.

3. M. Collinge, L. A. Burns-Naas, G. J. Chellman, T. T. Kawabata, W. J. Komocsar, J. R. Piccotti, J. Shenton and D. Wierda, Developmental immunotoxicity (DIT) testing of pharmaceuticals: current practices, state of the science, knowledge gaps, and recommendations, *J. Immunotoxicol.* **9** (2012) 210–230; <https://doi.org/10.3109/1547691X.2012.661486>
4. B. Blaszczyk, W. Lason and S. J. Czuczwar, Antiepileptic drugs and adverse skin reactions: An update, *Pharmacol. Rep.* **67** (2015) 426–434; <https://doi.org/10.1016/j.pharep.2014.11.009>
5. T. Shimada, T. Takemiya, H. Sugiura and K. Yamagata, Role of inflammatory mediators in the pathogenesis of epilepsy, *Mediators Inflamm.* **2014** (2014), Article 901902 (8 pages); <https://doi.org/10.1155/2014/901902>
6. E. E. de Vries, B. van den Munckhof, K. P. Braun, A. van Royen-Kerkhof, W. de Jager and F. E. Jansen, Inflammatory mediators in human epilepsy: A systematic review and meta-analysis, *Neurosci. Biobehav. Rev.* **63** (2016) 177–190; <https://doi.org/10.1016/j.neubiorev.2016.02.007>
7. J. T. Jarvela, F. R. Lopez-Picon, A. Plysjuk, S. Ruohonen and I. E. Holopainen, Temporal profiles of age-dependent changes in cytokine mRNA expression and glial cell activation after status epilepticus in postnatal rat hippocampus, *J. Neuroinflammation* **8** (2011), Article 29 (12 pages); <https://doi.org/10.1186/1742-2094-8-29>
8. R. Pacifici, L. Paris, S. Di Carlo, A. Bacosi, S. Pichini and P. Zuccaro, Cytokine production in blood mononuclear cells from epileptic patients, *Epilepsia* **36** (1995) 384–387.
9. G. De Sarro, D. Rotiroti, M.G. Audino, S. Gratteri and G. Nistico, Effects of interleukin-2 on various models of experimental epilepsy in DBA/2 mice, *Neuroimmunomodulation* **1** (1994) 361–369.
10. N. Basaran, F. Hincal, E. Kansu and A. Ciger, Humoral and cellular immune parameters in untreated and phenytoin-or carbamazepine-treated epileptic patients, *Int. J. Immunopharmacol.* **16** (1994) 1071–1077.
11. A. Basta-Kaim, B. Budziszewska, M. Leskiewicz, M. Kubera, G. Jagla, W. Nowak, S. J. Czuczwar and W. Lason, Effects of new antiepileptic drugs and progabide on the mitogen-induced proliferative activity of mouse splenocytes, *Pharmacol. Rep.* **60** (2008) 925–932.
12. H. Himmerich, S. Bartsch, H. Hamer, R. Mergl, J. Schonherr, C. Petersein, A. Munzer, K. C. Kirby, K. Bauer and U. Sack, Impact of mood stabilizers and antiepileptic drugs on cytokine production in-vitro, *J. Psychiatr. Res.* **47** (2013) 1751–1759; <https://doi.org/10.1016/j.jpsychires.2013.07.026>
13. H. Himmerich, S. Bartsch, H. Hamer, R. Mergl, J. Schonherr, C. Petersein, A. Munzer, K. C. Kirby, K. Bauer and U. Sack, Modulation of cytokine production by drugs with antiepileptic or mood stabilizer properties in anti-CD3- and anti-Cd40-stimulated blood in vitro, *Oxid. Med. Cell Longev.* **2014** (2014) article 806162 (11 pages); <https://doi.org/10.1155/2014/806162>
14. K. S. Prabhavalkar, N. B. Poovanpallil and L. K. Bhatt, Management of bipolar depression with lamotrigine: an antiepileptic mood stabilizer, *Front. Pharmacol.* **6** (2015) article 242 (11 pages); <https://doi.org/10.3389/fphar.2015.00242>
15. X. Q. Wang, B. Lv, H. F. Wang, X. Zhang, S. Y. Yu, X. S. Huang, J. T. Zhang, C. L. Tian and S. Y. Lang, Lamotrigine induced DIHS/DRESS: Manifestations, treatment, and outcome in 57 patients, *Clin. Neurol. Neurosurg.* **138** (2015) 1–7; <https://doi.org/10.1016/j.clineuro.2015.07.008>
16. S. Svalheim, U. Mushtaq, M. Mochol, G. Luef, M. Rauchenzauner, S. S. Froland and E. Tauboll, Reduced immunoglobulin levels in epilepsy patients treated with levetiracetam, lamotrigine, or carbamazepine, *Acta Neurol. Scand.* **127** (2013) 11–15; <https://doi.org/10.1111/ane.12044>
17. M. Fararjeh, M. K. Mohammad, Y. Bustanji, H. Alkhatib and S. Abdalla, Evaluation of immunosuppression induced by metronidazole in Balb/c mice and human peripheral blood lymphocytes, *Int. Immunopharmacol.* **8** (2008) 341–350; <https://doi.org/10.1016/j.intimp.2007.10.018>
18. E. K. St Louis, Monitoring antiepileptic drugs: a level-headed approach, *Curr. Neuropharmacol.* **7** (2009) 115–119; <https://doi.org/10.2174/157015909788848938>

19. E. Y. Abu-Rish, V. Kasabri, M. M. Hudaib, S. H. Mashalla, L. H. Al Alawi, K. Tawaha, M. K. Mo-hammad, Y. S. Mohamed and Y. Bustanji, Evaluation of antiproliferative activity of some tradi-tional anticancer herbal remedies from Jordan, *Trop. J. Pharm. Res.* **15** (2016) 469–474; <https://doi.org/10.4314/tjpr.v15i3.6>
20. G. Sachs, C. Bowden, J. R. Calabrese, T. Ketter, T. Thompson, R. White and B. Bentley, Effects of lamotrigine and lithium on body weight during maintenance treatment of bipolar I disorder, *Bi-polar Disord.* **8** (2006) 175–181; <https://doi.org/10.1111/j.1399-5618.2006.00308.x>
21. M. F. Cesta, Normal structure, function, and histology of the spleen, *Toxicol. Pathol.* **34** (2006) 455–465; <https://doi.org/10.1080/01926230600867743>
22. H. Tryphonas, Approaches to detecting immunotoxic effects of environmental contaminants in humans, *Environ. Health Perspect.* **109** (2001) 877–884.
23. R. Schrijvers, L. Gilissen, A. M. Chiriac and P. Demoly, Pathogenesis and diagnosis of delayed-type drug hypersensitivity reactions, from bedside to bench and back, *Clin. Transl. Allergy* **5** (2015) article 31 (10 pages); <https://doi.org/10.1186/s13601-015-0073-8>
24. M. C. Garcia-Pelayo, V. S. Bachy, D. A. Kaveh and P. J. Hogarth, BALB/c mice display more enhanced BCG vaccine induced Th1 and Th17 response than C57BL/6 mice but have equivalent protection, *Tuberculosis (Edinburgh)* **95** (2015) 48–53; <https://doi.org/10.1016/j.tube.2014.10.012>
25. E. Kay, L. Gomez-Garcia, A. Woodfin, R. S. Scotland and J. R. Whiteford, Sexual dimorphisms in leukocyte trafficking in a mouse peritonitis model, *J. Leukoc. Biol.* **98** (2015) 805–817; <https://doi.org/10.1189/jlb.3A1214-601RR>
26. D. J. Naisbitt, J. Farrell, G. Wong, J. P. Depta, C. C. Dodd, J. E. Hopkins, C. A. Gibney, D. W. Chad-wick, W. J. Pichler, M. Pirmohamed and B. K. Park, Characterization of drug-specific T cells in lamotrigine hypersensitivity, *J. Allergy Clin. Immunol.* **111** (2003) 1393–1403.
27. E. Beghi and S. Shorvon, Antiepileptic drugs and the immune system, *Epilepsia* **52** (2011) 40–44; <https://doi.org/10.1111/j.1528-1167.2011.03035.x>
28. J. M. Dwyer and C. Johnson, The use of concanavalin A to study the immunoregulation of human T cells, *Clin. Exp. Immunol.* **46** (1981) 237–249.
29. A. L. Rodríguez-Perea, E. D. Arcia, C. M. Rueda and P. A. Velilla, Phenotypical characterization of regulatory T cells in humans and rodents, *Clin. Exp. Immunol.* **185** (2016) 281–291; <https://doi.org/10.1111/cei.12804>
30. J. A. Bellanti, *Immunology IV: Clinical Applications in Health and Disease*, 3rd ed., I Care Press, Bethes-da (MD) 2012.
31. S. Liimatainen, M. Fallah, E. Kharazmi, A. M. Haapala, J. Isojarvi and J. Peltola, High concentra-tion of immunoglobulin A is associated with temporal lobe epilepsy, *Epilepsy Res.* **103** (2013) 54–61; <https://doi.org/10.1016/j.epilepsyres.2012.06.002>