Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

Ashur. M. M. Lmrabet\(^1\), Winarto Reki\(^2\), Edi Dharmana\(^3\), and Dwi Pudjonarko\(^4\)

\(^1\)Public Health department, Faculty of Medical Technology Msallata, Almergib University
\(^2,3,4\) Faculty of medicine, Diponegoro University

Email: ashur.almurabt@yahoo.com

Abstract

**Background:** Hepatitis B virus infection is a global concern health problem. Vaccination with Hepatitis B vaccine is the most efficient way to prevent the infection. Acute Stress may act as an effective adjuvant to increase the immune response to hepatitis B vaccine.

**Objectives:** To measure the impact of acute-stress on the immune response to hepatitis B vaccinated rats.

**Methods:** Pretest-Posttest control group design was conducted using animal models (24 Wistar Albino male rats). These were allocated into 4 vaccine groups: one period of acute-stress (X1), two periods of acute-stress (X2) and two groups control (C1, C2). Subsequent the adaption period, rats were given the hepatitis B vaccine.
Results: Data showed that two periods of acute-stress combined with two doses of vaccine led to an increase of macrophage number (%) and macrophage phagocytosis index (%). The macrophage number was \((0.51 \pm 0.038)\) in C2 and \((0.90 \pm 0.025)\) in X2, \((p = 0.001)\). The macrophage phagocytosis index was \((3.42 (3.21 – 3.90))\) in C2 and \((5.75 (5.57 – 6.81))\) in X2, \((p = 0.004)\). While, NK cells in vaccinated rats showed no response to acute stress.

Conclusion: Acute-stress acts as vaccine adjuvant and has positive effects, particularly on macrophage cells.

Keywords: Acute-stress, Macrophage Phagocytosis Index, Natural Killer cells.

Introduction

Hepatitis B Virus (HBV) infection is global health problem. Estimates by WHO that in 2015, 257 million persons in the world were living with chronic HBV infection. Therefore, administrating hepatitis B (hep-B) vaccine to people exposed to HBV is an essential preventative strategy. Notably, HBV infection is now a vaccine-preventable disease, accordingly; vaccination is the most primary prevention of the infection, as hep-B vaccine is safe and effective. Moreover, the efficacy of the vaccine is high; however, approximately 5-10% of healthy people are non-responders and they cannot induce the Hepatitis B surface antibodies (anti-HBs) using the standard vaccination scheme. As a result, there is a desperate need to improve vaccination efficacy in non-responders to protect them from HBV infection. Stress is a series of events comprising of a stressor (stimulus) that precipitates a reaction in the brain (stress perception) that in turn will activate the physiological fight response in the body (stress response). The characteristic of stress can be identified from its period of exposure; acute, which lasts either for a short period of time (minutes to hours) or chronic, which persists for several hours, a day, weeks, or even years. Various studies have found that acute stress (AS) may act as an effective adjuvant to the rat’s immune response, especially to vaccination. Vaccine administration may play a role as a behavioral adjuvant that enhances the...
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

Immune response in both animals and humans by the introduction of physical or mental stress\(^8\),\(^9\)\(^\) Therefore, designing therapeutic interventions involving behavioural manipulations, or administering cocktails of physiological mediators, might be increase the defence of immune system to fight against disease.\(^1\)\(^1\) This study used an animal model to investigate whether AS has a positive impact to the primary and secondary immune response to the hepatitis B vaccine, at the time of vaccine administration or at the first and second dose. Immune response was measured by using Phagocyte cells and NK cells. This study used the AS paradigm previously described and found to be effective in Wistar Albino rats. To our knowledge this is the first study investigated the impact of acute stress on immune response toward recombinant hepatitis B vaccine at the time of vaccine administration, and hypothesized that, acute-stress can increase macrophage number and phagocytosis index and increase of blood NK cells levels.

Materials and Methods

Twenty-four Wistar Albino male rats 8-10 weeks old with an average weight of 50.5 grams, were inbred in the animal laboratory of Gajah Mada University. The rats were housed in stainless steel cages in an air-conditioned house with the temperature maintained at 18\(^0\) C - 20\(^0\) C and 12:12 hours light-dark cycle and fed with normal pellets and tap water ad libitum, according to the Guideline for the Care and Use of Laboratory Animals in Faculty of Medicine Gadjah Mada University. The experiment was conducted with prior approval from Ethical Review Board Faculty of Medicine, Diponegoro University, with letter number 575/EC/FK-RSDK/2016. Rats were randomly allocated into four groups consisting of a group of rats exposed to AS once (X1), a group of rats exposed to AS two times (X2), and two control groups (C1, C2). All groups were adapted one week before the experiment started. Rats were immunized with intramuscularly 4µg recombinant hepatitis-B vaccine.

Vaccination Procedure:
All groups received intramuscular vaccination with recombinant hepatitis-B vaccine; one vaccination dose for control group1(C1) and intervention group1(X1), and two vaccination doses with interval of 4 weeks for control group 2 (C2) and intervention group 2 (X2). Each vaccine dose contained 4µg.\(^1\)\(^2\),\(^1\)\(^3\),\(^1\)\(^4\)

Acute- Stress Exposure:
Acute stress was administered according to the previous study.\(^1\)\(^1\),\(^1\)\(^5\)
Acute-stress was created by placing the subjects in well-ventilated wire
mesh restrainers for a single session of 3:00 hours from 9:00 AM - 12:00 PM, in the animal laboratory with the lights on. In order to verify the AS response, the Corticosterone hormone (CORT) hormone response was assessed immediately after 3 hours of AS exposure by drawing blood and measuring the hormone using ELISA assay.\(^{(16)}\) The reason for choosing this procedure was that such psychological stress mimics the experience in nature, especially the perception of confinement for rodents.\(^{(15),(17)}\) The psychological component of restraint stress is thought to arise from the mimicking a collapsed tunnel that is stressful for burrow-dwelling animals like rodents.\(^{(18)}\)

**Figure 1.** Wire-mesh restrainer. A= empty restrainer. B= rat inside the restrainer. The wire-mesh restrainer consists of a wooden base and stainless steel wire mesh restrainer hinged to the base. The dimensions are 13 cm long, 4 cm wide and 4 cm high. In addition, a door helped to secure the rat in the restrainer.

This type of stressor can activate the autonomic nervous system\(^{(19)}\) and the hypothalamic-pituitary-axis, and would activate adrenal steroid receptors in tissues throughout the rodent’s body.\(^{(11),(20),(21),(22)}\) The appropriate wire mesh size was determined before the start of the experiment.

**Blood collection**

Blood samples were collected from the retro-orbital sinus vein and kept in tubes without heparin (10 ml of blood). Samples were left to clot at room temperature for at least thirty minutes before they were centrifuged. Samples were centrifuged for 5 min at 2,500 rpm to separate the serum from the blood clott. The separated serum was then stored in a new eppendorf tube at -80\(^{0}\) C. All tubes were labeled accordingly and the ELISA procedure
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

immediately was performed according to manufacturers ELISA; kits insert (Rat CORT ELISA kit. Cataloge number: RC0073 (NeoScientific, USA) and Rat NK cell ELISA kit. Cataloge number:RN0030 (NeoScientific, USA).

Collection of macrophages and culture
Rats under anesthesia were placed on the dissecting table in a supine position, and the feet were fixed onto the board. Abdominal walls were disinfected with alcohol, and dissected aseptically using sterile instruments to expose the peritoneum. The peritoneum was disinfected with alcohol 70%, and then 20 mL cold RPMI solution was injected into the peritoneal cavity. And then massaged slowly to obtain more peritoneal macrophages. RPMI solution was aseptically aspirated with a pipette, and then put into a 15ml Falcon tube, centrifuged at 1,200 rpm at 4°C for 10 minutes. Erythrocyte contamination was rinsed out with Phosphate-Buffered Saline (PBS), 3% acetic acid was added for erythrocytes hemolysis several times to clean the solution. Supernatant was removed; 3mL of complete RPMI medium was added, consisting of RPMI 1640, FBS 10% (Fetal Bovine Serum) and penicillin, to make a pellet. A suspension was made again from the pellet with complete RPMI medium, counted with hemocytometer then resuspended to obtain 2.5 x 10^6 cells/mL. The cells were grown and cultured in complete medium in a covered 24 well micro-plate (flat base). Each well was filled with 200 uL with density 5 x 10^5 cell/mL, incubated with CO_2 at 37°C for 30 minutes and then 1mL RPMI complete medium was added to each well and further incubated for 2 hours. Cells were washed twice with RPMI, 1mL of complete medium added to each well and incubated for 24 hours. (23),(24)

Macrophage Phagocytic Index (Latex-Beads Method)
Macrophages were rinsed with RPMI two times, and cultured in 24 well micro-titre plate. Latex beads were made into suspension at a concentration of 2.5 x 10^7 /mL. Each well was filled with 200-uL latex/well, incubated for 60 minutes, CO_2 5% at 37°C. The cells were rinsed with PBS, to remove the un-phagocytized latex beads. A smear slide was made and fixed with absolute methanol. Slides were dried and stain with Giemsa 20% for about 30 minutes. Dyed smear slides were washed with distilled water and dried at room temperature. Object glass was put under the microscope. The phagocytosis activity was measured by counting the number of latex beads
phagocytized by 100 macrophage cells, the average number of latex positive cells is stated as the Macrophage Phagocytic Index.\(^{(23),(24)}\)

**Statistical analysis**

Statistical analysis used Statistical Program for Social Science (SPSS) v.21 (IBM Corp, USA). Data analyses consisted of descriptive analysis and test of the hypothesis. Descriptive analysis of variables expressed as mean ± standard deviation if normally distributed or median and range if a not normally distributed. Since the sample size was less than 50, data distribution was tested by the Shapiro-Wilk test.\(^{(25)}\) For CORT hormone variable; the normality test results obtained with the Shapiro-Wilk test for baseline and post AS exposure data for X1 and X2 (first exposure) showed a significance value which was greater than \(\alpha\) (0.05), meaning that the pre and post AS data of CORT level are normally distributed. One-way ANOVA test was used to determine the difference between groups. Then paired t-test was used to determine the differences between pre and post-AS exposure data for X1 and X2 groups. One-way ANOVA was repeated to see the difference between baseline data and first AS exposure and second AS exposure followed by Post Hoc test. For macrophage variables; normality test results with Shapiro-Wilk test showed significance greater than \(\alpha\) (0.05), meaning that the macrophage number data are normally distributed. The independent t test used to see the differences between X2 and C2 groups.

For latex level analysis, normality test results with Shapiro-Wilk test showed a significance value lower than \(\alpha\) (0.05), meaning that the data weren’t normally distributed. The Mann-Whitney test was used to see the differences between X2 and C2 groups.

For NK cell level; baseline data normality test results with Shapiro-Wilk test showed a significance value greater than \(\alpha\) (0.05), meaning that the baseline data are normally distributed, then One-way ANOVA test used to see the difference between groups. Normality test results with Shapiro-Wilk test for the post AS data showed a significance value lower than \(\alpha\) (0.05), meaning that the NK cell level post-acute stress data are normally distributed. The Kruskal-Wallis test used to see the difference between groups. The Wilcoxon test was used to see the differences between baseline data and post AS exposure.
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

Results

1. Acute Stress Exposure

Corticosterone hormone was used as the AS biomarker in this study, and the results are presented in (Table 1,2,3 and Figure 2,3).

<table>
<thead>
<tr>
<th>CORT level (ng/mL)</th>
<th>X1 (n=6)</th>
<th>X2 (n=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Pre-Acute-stress exposure</td>
<td>0.64 ± 0.025</td>
<td>0.63 ± 0.060</td>
<td>0.557&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-Acute-stress exposure</td>
<td>0.67 ± 0.138</td>
<td>0.71 ± 0.083</td>
<td>0.431&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: * significant difference  a - independent-test  b – paired - t-test

Corticosterone hormone levels increased in both, X1 and X2 groups. Therefore, CORT level significantly increased in X2 (intervention group 2) \( P < 0.05 \) (Table 1). The mean was 0.64 ± 0.025 ng/mL in the baseline data, while the mean was 0.67 ± 0.138 ng/mL in post AS exposure \( P = 0.639 \) for the X1 group. In the X2 group, the mean was 0.62 ± 0.060 ng/mL at baseline and 0.72 ± 0.083 ng/mL after AS exposure, \( p = 0.035 \).

![Figure 2. Box-plot mean of corticosterone hormone level after first AS exposure.](image)

The CORT hormone increased in both groups but, the increase was only significant in the X2 group \( p = 0.035 \).
Table 2. Corticosterone hormone levels of X2 group after AS exposure

<table>
<thead>
<tr>
<th>CORT level (ng/mL)</th>
<th>N</th>
<th>X2 Mean ± SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-AS exposure</td>
<td>6</td>
<td>0.62 ± 0.060</td>
<td></td>
</tr>
<tr>
<td>Post-AS exposure</td>
<td>6</td>
<td>0.71 ± 0.083</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-second AS exposure</td>
<td>6</td>
<td>0.78 ± 0.285</td>
<td></td>
</tr>
</tbody>
</table>

Note: * Significant; <sup>a</sup> Repeated ANOVA

Table 2 shows the CORT hormone level in X2 after first and second AS exposure.

CORT levels appear really different. The CORT levels were significantly increased \( P < 0.05 \) between the pre, first and second stress exposure in X2 group. \( P = 0.001 \). (Table 2 and figure 3)

Table 3. Post Hoc analysis of corticosterone hormone level of X2 after AS exposure

<table>
<thead>
<tr>
<th>CORT level Post Hoc test for X2</th>
<th>Pre-AS exposure</th>
<th>Post-first AS exposure</th>
<th>Post-second AS exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-AS exposure</td>
<td>–</td>
<td>0.035&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.212</td>
</tr>
<tr>
<td>Post-first AS exposure</td>
<td>0.035&lt;sup&gt;*&lt;/sup&gt;</td>
<td>–</td>
<td>0.556</td>
</tr>
<tr>
<td>Post-second AS exposure</td>
<td>0.212</td>
<td>0.556</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: * Significant

Table 3 shows that CORT level increased at first and second AS exposure. However, a significant increase \( P < 0.05 \) was observed after the second AS exposure compared with baseline data. \( P = 0.035 \).

Figure 3. Box plot of the mean corticosterone hormone levels in X2 group after AS exposure
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

2. Macrophage number and Latex level
Hypothesis AS can increase macrophage number and phagocyte index in vaccinated Wistar albino rats.

Table 4. Macrophage number and phagocytosis of latex

<table>
<thead>
<tr>
<th></th>
<th>C2 (N=6)</th>
<th>X2 (N=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage number %</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.51 ± 0.038</td>
<td>0.90 ± 0.025</td>
<td>0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phagocytosis of latex %</td>
<td>Median (Min–Max)</td>
<td>Median (Min–Max)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>3.42 (3.21 – 3.90)</td>
<td>5.75 (5.57 – 6.81)</td>
<td>0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: * Significant; <sup>a</sup> Independent t-test; <sup>b</sup> Mann-Whitney test

Based on Table 4 there were significant changes between the control group and intervention group p<0.05.

Figure 4. Macrophage number in X2 and C2 groups

Based on table 4 and figure 3, comparing C2 and X2 groups, there was a significant change in the macrophage number p<0.05. The mean increased from 0.51 ± 0.038 in C2 group to 0.90 ± 0.025 in X2 group p = 0.001. Furthermore, based on figure 3, the highest macrophage number was 93% in X2 group, and the lowest number was 47% in the C2 group.
Based on table 4 and figure 4, comparing C2 and X2 groups, there was a significant change in the latex level $p < 0.05$. The median increased from $3.42$ ($3.21$ – $3.90$) in the C2 group to $5.75$ ($5.57$ – $6.81$) in the X2 group $p = 0.004$. In table 4, the result of analysis of macrophage number and latex level showed a significant value, $p < 0.05$, so hypothesis $H_1$ was accepted.

3. Natural killer cell (NK) level

Hypothesis AS can increase the number of NK cells in vaccinated Wistar albino rats.

**Table 5. Natural killer cells in all groups after AS exposure**

<table>
<thead>
<tr>
<th>NK</th>
<th>C1 (N=6)</th>
<th>X1 (N=6)</th>
<th>C2 (N=6)</th>
<th>X2 (N=6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>0.091 ± 0.005</td>
<td>0.095 ± 0.007</td>
<td>0.091 ± 0.005</td>
<td>0.094 ± 0.003</td>
<td>0.560</td>
</tr>
<tr>
<td>Post</td>
<td>M (Min – Max)</td>
<td>M (Min – Max)</td>
<td>M (Min – Max)</td>
<td>M (Min – Max)</td>
<td>0.925</td>
</tr>
<tr>
<td></td>
<td>0.087 (0.024-0.200)</td>
<td>0.087 (0.078-0.100)</td>
<td>0.094 (0.076-0.300)</td>
<td>0.87 (0.077-0.300)</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0.893</td>
<td>0.042</td>
<td>0.600</td>
<td>0.917</td>
<td></td>
</tr>
</tbody>
</table>

Note: aOne Way ANOVA test; bKruskal-Wallis test; c Wilcoxon test

Based on Table 5, there were no significant changes $p > 0.05$ in baseline data between groups $p = 0.560$. Likewise, there were no significant differences $p > 0.05$ in post AS data between the groups $p = 0.925$. Further
testing used the Wilcoxon test to see differences between baseline data and post AS exposure. Based on table 5, there were no significant changes $p > 0.05$ in X2, C1 and C2 groups, respectively $p=0.917$ $p= 0.893$ $p = 0.600$. However, there were significant changes $p < 0.05$ in X1 group $p = 0.042$. There was no significant difference in the NK cell variables. Therefore, our results do not support the hypothesis H1.

**Discussion**

This study was designed to investigate whether an AS may have an effect as an adjuvant at the time of hep-B vaccine administration using macrophages numbers, macrophage phagocytosis and NK cell levels as parameters. The study investigated the effect of AS at first dose of vaccine (vaccine + AS) and the effect of AS at second dose of vaccine (vaccine + AS).

Serum corticosterone hormone was used as the AS biomarker. The elevated CORT hormone levels indicate that rats respond to AS exposure. As is known, there are two main neuroendocrine pathways activated in response to stress that control the immune system, namely the hypothalamic-pituitary-adrenal (HPA) axis which results in the release of CORT hormone, and the sympathetic nervous system which results in the release of catecholamine, epinephrine, and norepinephrine. AS is described as stress lasting over a period of minutes to hours. (9)

Restraint stress is thought to be largely psychological in nature, resulting from the perception of confinement on part of the animal. (15),(17) This stressor can activate the autonomic nervous system, and the HPA axis. (20),(21) In this study; rats were exposed to AS for three hours and blood were collected immediately after AS to measure CORT levels. It is likely that CORT hormone concentration levels decline during this time (three hours), as shown by previous studies that show that CORT hormone concentrations decline steadily over time. For instance, a study showed that the initial CORT levels were significantly higher but throughout the experimental period the CORT level was declined. (30) A similar study demonstrated that CORT hormone was significantly higher at 30 and 60 minutes than the controls, but not significant at 90 minutes. (31) Other studies showed that peak levels of CORT hormone in rats is at about 20 minutes following exposure to the stressor and then tend to fall off over the next 20 to 60 minutes. (32)-(33) Our study used male Wistar albino rats. However, studies have demonstrated that CORT hormone levels were higher in female than in male rats after acute stress exposure. (30),(34),(35)
On the other hand, in the X2 group the mean CORT hormone level increased significantly $p<0.05$, from $0.63 \pm 0.060$ ng/mL to $0.71 \pm 0.083$ ng/mL in post-AS exposure, $p=0.035$. This result indicates that CORT hormone is detectable after 3 hours of acute stress as stated above.

Macrophages are cells produced by differentiation of monocytes in tissue. (36) Macrophages’ role is detecting, engulfing and destroying pathogens (phagocytosis). After ingesting a microbe, a macrophage presents a protein on its cell surface called an antigen, this antigen is displayed on MHC class II molecule, which acts as a signal to other white blood cells, and then, T helper cells activate other cells of the immune system such as cytotoxic T cells to attack the infected cell. Macrophages also play a role in alerting the immune system to the presence of invaders and activate T helper cells; T helper cells start to stimulate the B cells (adaptive immunity) to secrete antibodies. In our results, acutely stressed rats shows higher macrophage numbers than nonstressed rats $p<0.05$. The mean increased from $0.51 \pm 0.038$ in C2 to $0.90 \pm 0.025$ in X2 $p=0.001$. Furthermore, acutely stressed rats demonstrated high latex level phagocytizes than nonstressed rats $p<0.05$. The median increased from $3.42$ ($3.21–3.90$) C2 to $5.75$ ($5.57–6.81$) in X2, $p=0.004$.

Our results are in line with many studies, which indicate that AS can enhance the immune system. AS induces a reorganization of leukocytes from the barracks, through the boulevards, and potentially to battlefield enhancing immune function. (10,37–40) Many studies that have focused on the alteration of lymphocyte functions and macrophage functions by AS, showed that acute cold-restraint stress increased phagocytic activity in peritoneal macrophages, (41) and cold swim stress results in the activation of peritoneal macrophages. (42)

It has been demonstrated that the phagocytic activity of rats, which had been exposed to restraint and fasting stress for 15 hours was increased. (43) Similar results from another study showed that restraint stress increased phagocytic activity of blood cells after 6 h. (44) A study conducted on mice showed that AS enhances the innate immunity by an increase the phagocytosis cell function. (45)

In the present study, three hours of wire-mesh restraint AS on Wistar albino rats increased the number of macrophage and macrophage phagocytic index of peritoneal macrophages. Thus, our results are consistent with previous studies where restraint AS can enhance the innate immune response by activating the macrophage.
Acute stress exposure did not show statistically significant changes in NK cell levels within groups, but in X1, there was a significant decrease between pre-and posttest data, \( p<0.05 \), (mean \( 0.095 \pm 0.007 \) ng/mL at baseline, and median \( 0.087 \) 0.078-0.100 ng/mL in post test data, \( p=0.042 \)).

Acute-stress can increase NK cells levels. For instance, psychological stress is associated with lymphocytosis-mobilization of a specific lymphocyte subtype that increases NK cells cytotoxicity. A meta-analysis found that NK cell levels rose significantly with AS.\(^{(46)}\) A study in which in 45 human subjects were exposed to short-term psychological stress (jumping by parachutists), examined plasma concentrations of cortisol and catecholamines continuously from 120 min before jumping to 60 min after. Lymphocyte subsets, NK cell numbers, and their respective functions were analyzed 2 hour before, immediately after and 1 hour after jumping. There was a significant increase in sympathetic-adrenal hormones during (adrenaline, noradrenaline) and shortly after jumping (cortisol). Lymphocyte subsets and the functional capacity of NK cells were enhanced immediately after jumping declining significantly to below baseline values 1 hr later. These changes correlated significantly with plasma concentrations of noradrenalin. Thus, rapid mobilization of NK cells has been suggested as a major mechanism be which the immune system adapts to stressful situations.\(^{(47)}\) Similar studies have reported that NK cell numbers and activity rise during physical exercise\(^{(48),(49)}\) and decrease after afterward.\(^{(50),(51)}\) based on these findings, the absence of significantly changes in NK cell levels in our study might be attributed to our use of the AS paradigm for 3 hours and the measurement at only one time-point after AS exposure, thus, NK cell levels might have increased and declined during AS.

In our opinion, the enhancement happened in the macrophage numbers and macrophage phagocytosis indicates that AS might play a positive role to support the immune function in the body. Our results are in agreement with other studies that indicate AS doesn't act as an immunosuppressant, and might act as immunoenhancement vaccine immune response. The current findings show that AS might act as a vaccine adjuvant and immunoenhancing effects of stress are particularly dramatic on innate immune (macrophages). Although the research has reached its aims, there were some limitations. According to several studies, steroid hormones production in AS occurs within 20-30 minutes. However, our study took the sample for steroid measurement at 3 hours, therefore the blood steroid levels may already have decreased.
In conclusion: Acute-stress significantly increased the macrophage number and macrophage phagocytosis. Meanwhile, AS shows no effects on the level of NK cells. Further research is needed to measure the effects of AS at earlier time points and to examine the effects of different time point, and using difference doses of vaccine. In addition, research is needed to explore the potential of using the physiological stress response in a clinically beneficial manner in vaccination program.

Acknowledgment

I would like to express my full appreciation to the staff of the Department of Scholarship, Ministry of Higher Education of Libya, for their support, both monetary and moral.

Declaration

We declared no conflict of interests.

References

7. Komatsu H. Hepatitis B virus: Where do we stand and what is the
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine


14. Li J. Adoptive transfer of immunity to hepatitis B virus by liver transplantation in rats. Diss Univ Duisburg-Essen, Medizinische Fak Univ Essen» Klin für Allg Visz Transplantationschirurgie. 2002;


16. Guéguinou N, Bojados M, Jamon M, Derradj H, Baatout S,


Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

29. Kvetfiansomsky R, Goldstein DS. and Release at Rest and during Immobilization Stress in Rats. Society. 1993;133(3).


45. Lyte M, Nelson SG, Thompson L. Innate and Adaptive Immune
Impact of acute stress on macrophage and NK cells in vaccinated Wistar albino rats with recombinant hepatitis-B vaccine

Responses in a Social Conflict Paradigm ’. 1990;


