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Effect of butylated hydroxyl toluene on the immune response of Rift Valley fever vaccine in a murine model

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ABSTRACT

The present study was planned to examine the effect of butylated hydroxy toluene (BHT) on the immune response of Rift Valley fever vaccine (RVFV) in Swiss mice. Animals were divided into four equal groups. The first group was kept as negative control. The 2nd group was orally administrated with the acceptable daily intake (ADI) of BHT 0.3 mg/kg b.wt. daily for 21 days and the 3rd group were vaccinated only by inactivated RVFV at a dose of 0.2 ml I/P two times. The 4th group was orally administrated BHT as in the 2nd group and vaccinated by inactivated RVFV as in the 4th group. Blood samples were collected from all groups two weeks from booster vaccination. The cellular immunity was determined by leucocytic indices and the neutrophil-lymphocyte ratio (NLR) whereas, humoral immunity was evaluated with IgG antibodies titer using enzyme-linked immune-sorbent assay (ELISA) test, serum neutralization test (SNT) and challenge test.

BHT induced leucopenia, neutrophilia and marked lymphocytopenia in both non-vaccinated and vaccinated mice. Moreover, BHT significantly decreased the efficiency of vaccination by inducing 70% cytopathic effect (CPE) in the infected cell cultures and increasing the ED_{50} value of RVFV vaccine. The present study indicates that BHT possesses a potential for decreasing both cellular and humoral mediated mechanisms.

1. Introduction

Preservatives extend the shelf-life of food and their different products by preventing their spoilage. Anti-oxidant is the most category of food preservatives as butylated hydroxy toluene (BHT) used widely in fatty containing foods [1]. The proper use of antioxidants depends on a basic understanding of the chemistry of fats and oil, mechanism of oxidation and function of an antioxidant in counteracting this type of deterioration [2]. BHT prevents the oxidation of oil and fats so prevent rancidity and development of off-flavor of margarine, candy food and flakes [3]. Several studies reported many side effects of BHT on the healthy condition of human and animals. It causes allergy, cancer of the nervous system, liver cancer and cirrhosis, hyperactivity and infertility [4–7].

BHT-induced oxidative stress in rats by decreasing the levels of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver and lung [8]. In addition, it had an immunosuppressive effect through reduction the cell activity in the natural killer (NK) of splenocytes [9].

Rift Valley fever (RVF) is a viral zoonotic disease distributed in tropical and Arabian countries. The virus is transmitted by an insect vector and replicated in host mammalian cells with a high incidence of lethality [10, 11]. Both DNA and attenuated vaccines have previously been shown to emphasize the specific humoral and cell-mediated immune responses to vaccination [12]. Due to the stability of BHT, it is used as an antioxidant in food products and infants milk to maintain the proper flavor and odor to prevent the oxidation of lipids [13]. As BHT had many sources other than foodstuffs, such medical uses in plastic syringe [14] and dental sealant placement [15] and water pollution from industrial purposes [16] thus, the overall exposure should be taken into account in the assessment of associated risks of BHT. The estimated daily intake (EDI) of BHT were significantly lower than ADI of this antioxidant established by the JECFA [17] as they estimated the level expected with consumed food only. The release of BHT, following dental sealant application in saliva significantly elevated the EDI in children to 6.51 mg/kg b.wt./day [15]. Consequently, the present study was conducted to determine the effect of ADI doses of BHT on both cellular and humoral immune responses against RVFV in a murine

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module to assess the possible hazard effect of BHT, especially on routine childhood vaccinations.

2. Materials and methods

2.1. Animals

Swiss mice weighing 20–25 g provided from the breeding unit of Holding company for biological products and vaccines (VACSERA) Giza – Egypt. Animals were provided with standard pellet food and had free access to drinking water. The institutional animal care and use committee (IACUC) of Cairo University properly approved the study protocol.

2.2. Chemicals

Butylated hydroxyl toluene (BHT): (E321) was supplied as an authentic powder from Sigma-Aldrich Fine Chemicals, St. Louis, MI, USA.

2.3. Rift Valley fever vaccine (RVFV)

It was obtained from the Rift Valley fever department of the holding company for biological products and vaccine (VACSERA) Ministry of health. The batch No. 160 used in this work was safe and potent according to the OIE & WHO requirements.

2.4. Baby Hamster kidney (BHK) cells

They were obtained from ATCC, USA. The cells were grown in tissue culture flask by were prepared using multiple extraction trypsinization technique [18].

2.5. Selection of dose

BHT antioxidant was last evaluated by JECFA when it was allocated on acceptable daily intake (ADI) of 0.3 mg/kg body weight based on a NOAEL of 25 mg/kg body weight/day from a two-year feeding study in the rat [17].

2.6. Animal group and dosing

Animals were divided into four groups with twenty-five mice in each as follow:

Group I, Distilled water (2 ml/kg b.wt.)

Group II BHT (0.3 mg/kg b.wt.) orally for 21 days.

Group III Distilled water (2 ml/kg) and vaccinated with inactivated RVFV at a dose of 0.2 ml intra-peritoneal (I/P) injection on the day 21 of the experiment and a booster dose after one week [19].

Group IV BHT (0.3 mg/kg b.wt.) for 21 days and vaccinated with inactivated RVFV at a dose of 0.2 ml I/P at the day 21 of the experiment and a booster dose after one week.

2.7. Blood sampling

All the animals were sacrificed after two weeks from the booster dose of vaccination and blood samples were collected for hematological and serological tests.

Two blood samples were collected one on EDTA for estimation of total leucocytic (TLC) count and differential leucocytic count (DLC). The other blood sample was collected in a sterile centrifugation tube without anticoagulant for separation of serum for serological tests. The serum samples were inactivated at 56 °C for 30 min to destroy most of its viral inhibitory activity.

Serum samples obtained were used for measurement of IgG, antibodies using direct enzyme-linked immune-sorbent assay (ELISA) test, serum neutralization test (SNT) test, and challenge test.

2.8. Neutrophil and lymphocyte indices

After the initial counts of TLC and DLC, blood samples were incubated with 80 mg/ml of nylon fibers for 10 min at 37 °C. The incubated blood samples were again analyzed for DLC. The product of the TLC and the percentages of neutrophil and lymphocyte known as the neutrophil and lymphocyte indices were determined for each of the respective groups [20].

2.9. Neutrophil-lymphocyte ratio (NLR)

The ratio is an acceptable immunological measurement of exposure to infection or vaccination in laboratory animals. It can be run without specialized equipment and is reliably potent to elude the most influencing factors [21].

2.10. IgG antibodies

The accurate quantitation of the IgG (Y-chain) antibodies in the serum may be achieved using the direct ELISA test [22]. The virus-specific IgG present in the serum will bind to the viral antigens using Horse reddish peroxidize (HRP) that reacts with ABTS substrate to produce specific color that detected colorimetrically at 405 nm.

2.11. Serum neutralization test (SNT) and cytopathic effect (CPE)

The serum-virus neutralization assay is a highly specific serological test to detect the presence and magnitude of functional systemic antibodies that prevent infectivity of a virus. The assay is a highly sensitive and specific test that to measure the titer of neutralizing antibodies post-vaccination [23]. The two-fold diluted sera were incubated with diluted antigen in tissue culture microstate plates that are contained BHK monolayer and examined daily under the inverted microscope for detection of cytopathic effect (CPE).

2.12. Challenge test

New two groups of weaning mice 60 animals each were divided into control and BHT-treated (21 days) were inoculated with five-fold serial dilutions inactivated RVFV (undiluted, 1/5–1/25–1/125–1/625). The groups were challenged after two weeks from booster doses using RVF challenge virus at 1000 LD_{50}/ml at a dose of 0.2 ml I/P. The numbers of dead and alive mice were calculated according to the method of Reed and Muench [24], and results were expressed using the reciprocals of the dilutions.

2.13. Statistical analysis

All the values were expressed as mean ± SEM using (S.E.). The data were subjected to student *t*-test using the statistical analysis software (SPSS) Ver. 15, under Windows XP. Comparisons between groups were used to determine the statistical significance between various groups. Differences were considered to be statistically significant when $*p < 0.05$ and $**p < 0.01$.

3. Results

3.1. Total and differential leucocytic counts

BHT at a dose of 0.3 mg/kg b.wt. daily for 3 weeks induced leucopenia, neutrophilia and marked lymphocytopenia in both non-vaccinated and vaccinated mice when compared to group I and group III (Table 1). The differences were highly statistically significant at $p < 0.001$.