Zagazig Veterinary Journal
Volume 43, Number 3, p. 145-151, 2015

DOI: 10.21608/zvjz.2015.28451

Interaction of Ceftriaxone with Echinacea

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Article History: Received: 15/10/2015 Received in revised form: 24/11/2015 Accepted: 31/12/2015

Abstract
Ceftriaxone is a third-generation cephalosporin with a broad-spectrum activity against Gram-positive and Gram-negative bacteria. Ceftriaxone induced lipid peroxidation which generated the peroxidases and hydro peroxidases. The aforementioned substances caused an immune suppression. *Echinacea* is a popular herbal product in North America and Europe, used as an immune-modulatory product. The objectives of this study was to evaluate the effects of Echinacea, ceftriaxone and their combination on some immunological parameters in vaccinated rabbits, moreover to investigate the effect of Echinacea to ameliorate the side effect and immune-suppression of ceftriaxone. The experiment was carried out on twenty-five male New Zealand white rabbits .They were divided into 5 equal groups, each consisting of five rabbits (control, vaccinated with formalized killed *P.multocida* vaccine, vaccinated and treated with 50 mg/Kg BW Ceftriaxone I/M once daily for 5 days, vaccinated and treated with 50 mg/Kg BW Echinacea orally once daily for 5 days and vaccinated and treated with Ceftriaxone and Echinacea). Some immunological parameters had been estimated (Nitric Oxide, Lysosomal activity, Lymphocyte transformation and fractionation of serum protein. The Echinacea group was the best in all estimated parameters, while the Ceftriaxone group was the worst. Both Echinacea and ceftriaxone in a combination improved the immunological parameters. In ceftriaxone group, serum nitric oxide and serum lysozyme activity were 6.73±0.32um/ml and 139.60±2.29Ugm/ml; 6.62±0.21um/ml and 136.00±1.14Ugm/ml; 6.33±0.23um/ml and 130.80±1.02Ugm/ml at the 1st, 2nd and 3rd day post vaccination, respectively. The immunological parameters were better in the combined group (14.78±0.15 γ-globulin, 14.62±0.16 β-globulin, 11.96±0.10 α-globulin, 62.09±0.05 albumin and 0.89±0.03 Lymphocyte Transformation index) when compared with the ceftriaxone group (10.22±0.17 γ-globulin, 13.13±0.16 β-globulin, 10.04±0.39 α-globulin, 60.55±0.53 albumin and 0.59±0.02 Lymphocyte Transformation index). Therefore, the current study concluded that combination of both ceftriaxone and Echinacea have a promising protective action and improving rabbit immunity, through ameliorating the obvious immune-suppression of the Ceftriaxone alone.
Keywords: Ceftriaxone, Echinacea, Nitric oxide, Lysozyme

Introduction

Exploratory studies of ceftriaxone revealed an induction of lipid peroxidation which generated the peroxidases and hydro peroxidases. The aforementioned substances caused an immune suppression [1]. *Echinacea* describes a genus of perennial plants native to the prairies of mid-western North America [2]. Currently the medicinal use of *Echinacea* is based on its immune-modulatory properties. It is mainly used to treat and prevent upper respiratory tract infections, such as common cold and influenza [3,4].

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Stemmed from the previous notion, this study was designed to evaluate the effects of Echinacea, ceftriaxone and their combination on some immunological parameters in vaccinated rabbits, moreover to investigate the effect of Echinacea to ameliorate the side effect and immune suppression of ceftriaxone in formalized killed *P.multocida* vaccinated rabbits.

Material and Methods

Experimental animals

Twenty-five male New Zealand white rabbits weighing 1550–1600 g were collected from the laboratory Animal House, Faculty of Veterinary Medicine, Zagazig University. All animals were kept for 2 weeks before starting the experimental study for acclimatization. They were fed on standard diet of commercial pellets all over the experimental period and housed in batteries with good hygienic conditions.

Experimental design

Twenty-five male New Zealand white rabbits were divided randomly into 5 equal groups, each consisting of 5 rabbits. They were fed on *ad-libitum* standard diet of commercial pellets. Control group: none vaccinated and none treated; 2nd group**:** rabbits were vaccinated with formalized killed *P. multocida* vaccine (4×10^9/ml CFU of *P. multocida*) 1 ml S/C 3rdgroup: rabbits were vaccinated and treated with ceftriaxone (Ceftriaxone Sandoz®) 50 mg/Kg BW I/M injection once daily for 5 successive days [5]; 4th group: rabbits were vaccinated and treated with Echinacea (Mulone®) 50 mg/Kg BW orally once daily for 5 successive days [6]; 5thgroup: rabbits were vaccinated and treated with ceftriaxone 50 mg/Kg BW I/M injection once daily for 5 days with Echinacea 50 mg/Kg BW orally once daily for 5 successive days. Rabbits of groups (2-5) were vaccinated on the 5th day from the beginning of the treatment. Two blood samples were collected from the ear vein of all groups on 1st, 2nd, 3rd and 7thdays post vaccination. The first was used to separate the serum and the 2nd was collected with anticoagulant (EDTA).

Nitric Oxide production assay was performed on 1st, 2nd and 3rd days post vaccination according to Sun *et al.* [7]. Lysosomal activity was performed on 1st, 2nd and 3rd days post vaccination according to the method described by Mohrig and Messner [8]. Lymphocyte transformation index was performed on 3rd day post vaccination according to the technique described by Rai-El-Balhaa *et al.* [9]. Fractionation of serum protein using Sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) technique was performed on 7th day post vaccination. Qualitative fractionation of serum protein to determine the serum albumin, alpha, beta and gamma-globulins were carried out using polyacrylamide gel columns according to the technique described by Davis [10] and Ornstein [11].

Statistical analysis:

Data were collected, organized and analyzed using one-way analysis of variance (ANOVA) through the general linear models (GLM) procedure of the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA) .The comparison of means was carried out with Duncan's multiple range tests. Results were recorded as mean ± standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance.

Results

There was a significant ($P \leq 0.05$) elevation in serum Nitric Oxide level in the formalized killed *Pasteurella multocida* vaccinated group and the Echinacea treated groups, while ceftriaxone and its combination with Echinacea group showed significant ($P\leq 0.05$) decrease on 1st and 2nd days post vaccination. Moreover, on the 3rd day post vaccination, vaccinated group showed non-significant increase, while the combined group showed non significant reduction. Ceftriaxone group revealed a significant ($P\leq 0.05$) decrease, while Echinacea group showed a significant ($P\leq 0.05$) increase in serum Nitric Oxide level (Table 1).

Table 1: Effect of Ceftriaxone (50 mg/Kg BW I/M), Echinacea (50 mg/Kg BW orally) and their combination with the same doses on serum nitric oxide production (um/ml) and lysozyme activity (Ugm/ml) in male rabbits.

Parameters		Groups				
	DPV*	Control	Vaccinated**	Ceftriaxone	Echinacea	Echinacea+Ceftriaxone
Serum nitric oxide	1st	9.18±0.37^{c}	16.14±1.21^{b}	6.73±0.32^{d}	23.87±0.69^{a}	6.49±0.33^{d}
	2nd	9.18±0.31^{c}	14.05±1.02^{b}	6.62±0.21^{d}	20.17±0.71^{a}	7.81±0.27^{d}
	3rd	9.20±0.35^{b}	10.05±0.92^{b}	6.33±0.23^{c}	15.94±0.47^{a}	8.97±0.27^{b}
Serum lysozyme activity	1st	161.00±1.52^{c}	169.00±1.79^{b}	139.60±2.29^{d}	187.80±3.89^{a}	137.00±2.59^{d}
	2nd	153.60±1.72^{c}	159.20±1.56^{b}	136.00±1.14^{d}	178.60±2.82^{a}	140.80±2.65^{d}
	3rd	151.00±1.64^{b}	154.40±2.62^{b}	130.80±1.02^{c}	171.00±2.26^{a}	150.00±3.35^{b}

*DPV: day post vaccination; ** Vaccinated only; Means with different superscripts within the same row were significant different at P< 0.05.

Results showed significant (P≤ 0.05) elevation in serum lysozyme activity in the formalized killed *Pasteurella multocida* vaccinated group and the Echinacea treated group, while ceftriaxone and its combination with Echinacea group showed significant (P≤0.05) decrease on 1st and 2nd days post vaccination. Moreover, on the 3rd day post vaccination, vaccinated group showed non-significant increase, while the combination group showed non significant decrease. Ceftriaxone group revealed a significant (P≤0.05) decrease, while Echinacea group showed a significant (P≤0.05) increase of serum lysozyme activity (Table 1). Ceftriaxone treated group revealed a significant decrease in lymphocyte transformation. On the contrary, Echinacea treated group showed a significant increase in lymphocyte transformation, while the combination group showed non significant decrease in lymphocyte transformation (Table 2).

The vaccinated group revealed a significant increase in all globulin fractions and albumin level compared with the control group. Ceftriaxone treated group denoted a significant decrease in all globulin fractions and albumin level. On the other hand, Echinacea treated group exhibited a significant increase in all globulins fractions and non significant increase in albumin level. The combination group showed non significant increase in albumin, alpha and beta globulins with a significant increase in the gamma fraction (Table 2).

Table 2: Effect of Ceftriaxone (50 mg/Kg BW I/M), Echinacea (50 mg/Kg BW orally) and their combination with the same doses on protein fractions using electrophoresis technique and Lymphocyte Transformation index in male rabbits.

Fraction	Group				
	Control	Vaccinated only	Ceftriaxone	Echinacea	Echinacea+Ceftriaxone
γ-globulin	12.28±0.09^{c}	13.75±0.11^{b}	10.22±0.17^{d}	16.64±0.20^{a}	14.78±0.15^{b}
β-globulin	14.44±0.12^{c}	14.89±0.18^{ab}	13.13±0.16^{d}	15.00±0.06^{a}	14.62±0.16^{ac}
α-globulin	11.33±0.10^{b}	12.13±0.16^{a}	10.04±0.39^{c}	12.63±0.13^{a}	11.96±0.10^{ab}
albumin	61.95±0.14^{b}	62.89±0.17^{a}	60.55±0.53^{c}	62.18±0.13^{ab}	62.09±0.05^{ab}
LTI*	0.90±0.02^{b}	1.07±0.02^{b}	0.59±0.02^{d}	1.24±0.03^{a}	0.89±0.03^{b}

*LTI: Lymphocyte Transformation index; Means with different superscripts within the same row were significant different at P < 0.05.

Discussion

Echinacea purpurea is best known for its effect on the immune system [12]. Stimulation of various immune cells such as macrophages, monocytes, and natural killer (NK) cells had been demonstrated repeatedly in vitro [13]. Ceftriaxone has a long half-life allows for once-daily dosing, making ceftriaxone an excellent drug for outpatient therapy of community-acquired infections. Ceftriaxone is also useful for the treatment of Lyme disease, interperitoneal and sexually transmitted diseases [14].

In the present investigation, rabbits vaccinated with formalin killed *Pasteurella multocida* vaccine induced a significant increase in serum nitric oxide and lysozyme levels on the 1st and 2nd days post vaccination assuring the efficacy of this vaccine in rabbits but revealed non-significant increase in both parameters besides the lymphocyte transformation ratio in the 3rd day. Results also showed significant increase in gamma fraction of globulins on the 7th day post vaccination. The present data were in accordance with Hussaini *et al.* [15] who reported that pooled serum sample from rabbits vaccinated with live and killed form of the clone showed 66% protection while active immunization with the recombinant clone conferred 83% immunity to rabbits when challenged with a lethal dose of *P. multocida*. ELISA results were positive for the presence of antibody in serum of immunized rabbits. Rabbit alveolar macrophages stimulated by the injection of killed Bacillus Calmette Guérin tuberculosis vaccine exhibited an increas in the levels of various hydrolytic enzymes, including lysozyme activity and Nitric Oxide after 6, 12, 18 and 24 hours post exposure, as compared to alveolar macrophages from un-stimulated rabbits [16].

In the present study, oral administration of Echinacea 50 mg/Kg BW presented a significant increase in serum Nitric Oxide level on the 1st, 2nd and 3rd days post vaccination. These results were in accordance with that reported by Chen *et al.* [17] who demonstrated the stimulating effects of Echinacea extracts and certain individual fractions on production of Nitric Oxide and TNF in an activated macrophage cell line after 7 hours of exposure indicating the rapid action of its constituents on macrophage activity.

In this study I/M injection of Ceftriaxone 50 mg/Kg BW elicited a significant decrease in serum NO level on the 1st, 2nd and 3rd day post vaccination due to persistent inhibition of NO production. Microglia is the major inflammatory cells in the central nervous system and activated in response to brain injuries. Moreover, activated microglia is known to release a variety of pro inflammatory cytokines such as nitric oxide (NO). Kim *et al.* [18] studied the inhibitory effects of ceftriaxone on NO production observed in lipopolysaccharides (LPS)-challenged BV2 murine microglial cells. Further, effects of ceftriaxone on inducible nitric oxide synthase (iNOS) expression levels were also determined. The results showed that ceftriaxone significantly inhibited NO production and iNOS expression in BV2 microglial cells after 9 hours post exposure. These findings were supported by estimation of 4-HNE (4-hydroxynonenal) content as peroxidation marker suggesting that ceftriaxone should be evaluated as potential therapeutic agent for meningitis but showed immunosuppressive action through inhibition of NO release due to the excessive activation of lipid peroxidation and free radicals specially hydroperoxidases and harmful endproducts.

In the current study, oral administration of Echinacea 50 mg/Kg BW for 5 days exhibited a significant increase in serum lysozyme activity on the 1st, 2nd and 3rd day post vaccination. To date, only a few reports have yet studied the effects of *Echinacea* on the production and secretion of lysozyme activity, the important molecule of the innate immune response. Helal [19] studied the effect of *Echinacea purpurea* root extract (standardized to 7.84% polysaccharides) on the production and secretion of lysozyme activity in the human monocytic cell line THP1 and in the epithelial cell line HT-29. The results showed that *Echinacea* root extract induced significant increase in the secretion of lysozyme activity of THP1 and epithelial cells after one hour of exposure.

In this study, it was found that oral administration of I/M injection of Ceftriaxone 50 mg/Kg BW for 5 days elicited a significant decrease in serum lysozyme level on the, 1st , 2nd and 3rd days post vaccination. The mechanism underlying the inhibition effect of ceftriaxone remains to be elucidated. In particular, neutralization of the medium by its acidification properties significantly reduces the lysozyme exocytosis by ceftriaxone, also

inhibition of NADPH oxidase needed for activation of lysosomes [20].

In the current study, oral administration of Echinacea 50 mg/Kg BW for 5 days exhibited a significant increase in the lymphocyte transformation index on the 3rd day post vaccination. The data was in agreement with Diana *et al.* [21] they investigated the effects of Echinacea (50 mg/kg of aerial parts) on phagocytic activity, interleukin (IL-2) levels and lymphocyte blastogensis and proliferation in 12-month-old, healthy male Sprague Dawley rats when administered over one week period. Echinacea significantly increased number of lymphocyte and its blastometric ratio. Due to activation of lymphocyte transforming factor (LTF) a lymphokine causing transformation and clonal expansion of non-sensitized lymphocytes.

In the current study, I/M injection of Ceftriaxone 50 mg/Kg BW for 5 days exhibited a significant decrease in lymphocyte transformation index on the 3rd day post vaccination. Result which was similar to that of Bruno *et al*. [22] who studied the aspects of lymphocyte functions. In order to quantify and compare immunological properties of antibiotics, calculated an "immune index" which defined as: the number of positive statements-number of negative statements/total number of statements. One of these statements was lymphocyte proliferation. Ceftriaxone assured antiproliferative effect on lymphocyte due to its inhibitory effect on mitogen PHA (phytohaemagglutinin) leading to decreased transformation rate.

In this study, oral administration of Echinacea 50 mg/Kg BW for 5 days exhibited a significant increase in gamma globulins concentration on the 7th day post vaccination. Similar results were observed by Ahmed *et al.* [23] who studied the effect of Echinacea purpurea extract supplementation on growth performance and immuno-biochemical traits of growing rabbits. The first group was served as the control, whiles the second was treated orally with 50 mg Echinacea purpurea extract/kg BW daily. They showed that *Echinacea extract* stimulates immune functions as evidenced by the significant increase in gamma globulins percentage which may be due to its basic content of polysaccharides which are primary active ingredients for immune modulating effects and polyclonal activation [24].

In the current study, I/M injection of Ceftriaxone 50 mg/Kg BW for 5 days exhibited a significant decrease in the gamma globulins concentration on the 7thday post vaccination. Studies not only approved high binding of ceftriaxone to albumin as carrier protein but also ceftriaxone formed a conjugate with proteins due to presence of hydrophobic free polar group [25]. The mentioned finding were well supported by Brouwers *et al.* [26], they investigated the electrophoretic properties of ceftriaxone on protein fractions mobility and concentrations . Ceftriaxone slowed down migration of all fraction with special statistical reduction in γ-globulin concentration due to drug power in containing reactive group which react with drug receptors probably take a role in electrophoretic mobility and concentration of all protein fractions.

Conclusion

In conclusion, the use of *Echinacea* and Ceftriaxone in the treatment of bacterial diseases augments the immune response through protective effects against the pronounced immunosuppressive effects produced by Ceftriaxone alone in mono-therapy manner.

Conflict of interest

None of the authors have any conflict of interest to declare

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الملخص العربى

التداخل الدوائى بين السيفترياكسون و الاكينسيا

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السيفترياكسون هو واحد من الجيل الثالث لمجموعات السيفالوسبورين. وهو أيضاالدواء الامثل لعلاج بعض الحالات الحرجه. أصبحت الاكينيسيا واحدة من أشهر النباتات الطبية في أمريكا الشمالية وأوروبا كمحفز للمناعة, خاصة لمنع وعلاج عدوي الجهاز التنفسي العلوي. اجريت هذه الدراسة لمقارنة تأثير كلا من السيفترياكسون و الاكينيسيا كلا على حده والخليط منهما على الاستجابة المناعية فى الارانب البيضاء المحصنة بلقاح الباستيريلا مالتوسيدا. تم تقسيم الارانب (٢٥ ارنبا) إلى ٥ مجموعات خمسه ارانب فى كل مجموعة. المجموعة الأولى : الضابطة والمجموعة الثانية: المحصنه: تم تحصينها بالباستيريلا مالتوسيدا, المجموعة الثالثه: (محصنة ومعالجة بالسيفترياكسون (٥٠مجم/ كجم) عن طريق الحقن بالعضل لمدة خمسة أيام على التوالى).والمجموعة الرابعه: (محصنة ومعالجة بالاكينيسيا) (٥٠مجم/ كجم) عن طريق الفم لمدة خمسة أيام على التوالى،المجموعة الخامسة: (محصنة ومعالجة بخليط السيفترياكسون و بالاكينيسيا). وتم اجراء بعض الاختبارات المناعيه مثل نشاط الليزوزيم- أنتاج أوكسيد النيترويك-تحول الخلايا اللمفاوية- فصل البروتين(نسبه الجاما جلوبيولين):- اظهرت المجموعتين المحصنه فقط ومجموعه الاكينسيا نقاط مميزه وايجابيه فى كل القياسات. بينما على العكس, مجموعه السيفترياكسون اظهرت نقاط سلبيه فى جميع القياسات. والمجموعه المعطاه الخليط بين السيفترياكسون والاكينسيا اظهرت نتائج مبشره وقائيه للمناعه فى جميع القياسات. من كل ما سبق نستخلص امكانيه استخدام الاكينسيا مع السيفترياكسون في علاج

الامراض البكتيرية بهدف تقوية الاستجابة المناعية وتقليل الاثار الجانبيه والمثبطه للمناعه التى يحدثها السيفترياكسون كعلاج منفرد.