HYDROGEN PEROXIDE EFFECTS ON IMMUNE RESPONSES (CELLULAR AND HUMERAL) IMMUNITY OF ADULT MALE RABBITS

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Received 11/6/2008 Accepted 3/12/2008

ABSTRACT

This study was designed to investigate the effects of 0.5% hydrogen peroxide H_2O_2 and levamizole drug on immune response of adult male rabbits. Twenty four mature male newzeland were randomly divided into two groups and were treated as follow for four weeks: Control group(C) and H_2O_2 treated group (GI) which were daily intubated with 0.5% H_2O_2 in drinking water. After cessation of H_2O_2 intubation (4weeks), animals of GI group where intubated (each 72 hrs) with levamizole (5mg /Kg B.W.) in drinking water for another four weeks . Blood samples were taken at zero, 4 and 8 weeks of the experiment to determine some blood and immune response parameters including: differential leukocytes count, total leukocytes count (TLC), active and T- lymphocytes, neutrophil/lymphocyte (N/L) index, platelets count , phagocytic activity and IgG concentration. The results conducted that H_2O_2 treated group showed significant decrease ($p<0.05$) in some blood and immune response parameters manifested by leucopenia, lymphocytopenia, thrombocytopenia and a significant increase ($P <0.05$) in N/L index with significant suppression in the percentage of active and total lymphocyte, phagocytic activity and serum IgG concentration. On the other hand, levamizole treatment leading to a significant increase ($P<0.05$) in the immune response parameters and a significant decrease (P0.05) in N/L index.

Key words: H_2O_2, IgG, Immune response, ROS.

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تأثيرات بيرو كسيد الهيدروجين على الاستجابة المناعية(الخلطية و الخلوية) في ذكور الأرانب البالغة

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الاستلام 2008/6/11 القبول 2008/12/3

الخلاصة

صممت هذه التجربة لمعرفة تأثير بيرو كسيد الهيدروجين بتركيز0.5% وعقار الليفاميزول على الاستجابة المناعية في ذكور الأرانب البالغة. تم استخدام 24 أرنباً بالغاً، قسمت عشوائيا إلى مجموعتين متساويتين وعوملت لمدة 4 أسابيع كالآتي: أعطيت المجموعة الأولى(السيطرة -C) ماء الشرب الاعتيادي؛ أما المجموعة الثانية (G) فقد أعطيت ماء الشرب الاعتيادي مضافا" إليه بيرو كسيد الهدروجين بتركيز 0.5 % ثم جرعت حيوانات هذه المجموعة بعقار الليفاميزول (كل 72 ساعة) وبجرعة(5 ملغم / كغم من وزن الجسم) لمدة أربعة أسابيع آخرى و تم بعد ذلك سحب عينات الدم للأسابيع 0 ،4،8 من التجربة لغرض إجراء الفحوص المناعية الآتية : العدد الكلي والتفريقي لخلايا الدم البيض و النسبة المئوية للخلايا اللمفاوية التائية (الفعالة والكلية)و معامل الخلايا العدلة/اللمفاوية و أعداد الأقراص الدموية و فعالية البلعمة وتركيز الكلوبيولين المناعي G . لقد أظهرت النتائج حدوث انخفاض معنوي (P<0.05) في المعايير المناعية تمثلت بانخفاض في أعداد خلايا الدم البيض والخلايا اللمفاوية والأقراص الدموية مع حدوث ارتفاع معنوي في معامل الخلايا العدلة /الخلايا اللمفاوية وانخفاض في النسبة المئوية للخلايا اللمفاوية التائية (الفعالة والكلية)، فضلاً عن حدوث انخفاض في معامل البلعمة وتركيز الكلوبيولين المناعي IgG في مصل الدم. كما أشارت النتائج إلى الدور المناعي لليفاميزول تمثلت بحدوث ارتفاع معنوي في جميع المعايير المناعية المدروسة (P<0.05) وانخفاض في معامل الخلايا العدلة/اللمفاوية. يستنتج من هذه الدراسة إلى دور الليفاميزول المتمثل بحدوث ارتفاع معنوي بجميع معايير المناعة .

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INTRODUCTION

Reactive oxygen species (ROS) including radical species such as superoxide anion, hydroxyl radical, singlet oxygen and non radical species, such hydrogen peroxide can be generated in vivo by exogenous factors such as imbalance in diet, tobacco, smoke, exposure to strong pollutant or an oxidant or from endogenous sources such as lipid peroxidation, inflammation, secondary lesions and biochemical reaction (1,2,3). Besides, free radicals (FRs) are produced by the cells of immune system as weapons against foreign invader, where generation of ROS, product of oxidant burst, represent an essential elements for microbial killing (4, 5, 6, 7). At physiological concentration endogenous ROS help to maintain homeostasis, and there is delicate balance between the amount of FRs production in the body and the antioxidant needed for provide protection against them (8,9,10,11), however, on excess FRs production for prolonged period of time or lack of antioxidant defences can shift this balance resulting in a state of oxidative stress with adverse tissue damage (12,13,14).

Different stress condition (nutritional, environmental, viral and bacterial infections) is associated with over production of ROS (15, 16, 17, and 18). Besides, abundance of FRs, appear to be produce as side effect of immune cell activation (19,20). Immune cell contain higher concentration of polyunsaturated fatty acids (PuFA), which are vurnable to oxidation by FRs resulting to various type of tissue damage in and within the cell with subsequent depression in the immune system function (21,22). An increase in ROS, to which H_2O_2 is belonged, can trigger activation of stress sensitive proteins signalling pathway or inflammatory activated kinases, such as JNK (stress protein) which may play an important in the pathogenesis of oxidation strees –induced immunosuppressant effect (23,24).

Pi and his coworkers (25) revealed that provision to exogenous H_2O_2 or dimethyl malate resulted in raises intracellular level of H_2O_2 leading to disturbing in metabolic signals for insulin secretion, hyperglycemia, glucose toxicity, lipid and protein glycation with subsequent decrease in immunity.

On conclusion, there are two sides of immune system and FRs, although the germicidal role of H_2O_2 is well established, a hypothetical regulatory functions either promote or inhibit immunity is still controversial. To these aims, the investigation is dedicated.

MATERIALS AND METHODS

Twenty four mature (four to six months old) adult Newzeland male rabbits were randomly divided into two groups (each of 12), group C , they had free access to food and water and served as control, group I (GI), animals in this group were subjected to ad libitum supply of drinking water containing 0.5% H_2O_2 (35% of hydrogen peroxide solution were diluted 70 times), then after four weeks, groupG1 were subjected to oral intubation of (5mg/kg B.W.) of levamizole each 72hr. for four weeks. Blood samples were collected by heart puncture technique at 0, 4, and 8 weeks of the experiment for measuring the following parameters:

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Blood sample were used immediately for measuring the percentage of T-lymphocyte by Erythrocyte-rosette test as described by Braganza and his coworkers (26), total white blood cells (TLC), the percentage of WBC differential count, platelet count as described by Johnstone and Robin (27).

Neutrophil / lymphocyte (N/L) index was measured as described by Campell (28).While phagocyte activity (%) was measured according to Metcalf and his coworkers(29). Besides , serum sample were collected for measuring immunoglobulin G (IgG) ng/ml by Enzyme Linked Immuno Sorbent Assay (ELISA) as described by Newkirk and his coworkers(30).

Differences between experimental groups were evaluated using two-way analysis of Variance (ANOVA) .Specific group differences were determined using least significant differences (LSD). For all analysis, a P value 0.05 was considered to be significant (31).

RESULTS

The effect of 0.5% H_2O_2 and levamizole on cellular and humeral immunity of male rabbits was shown in tables (1, 2, 3, and 4). Table (1) showed percentage of N/L index and differential leukocyte count in normal and H_2O_2 treated rabbits. The results revealed that exposure of animals to H_2O_2 for four weeks produce significant decrease ($P<0.05$) in percentage of lymphocyte comparing to control. A significant increase in this parameter was observed after levamizole treatment in H_2O_2 treated group (GI) comparing to pretreated period. Non significant ($P>0.05$) differences were observed in monocyte and neutrophil percentage in G I and control before treatment with levamizole. While treatment with levamizole significantly increase monocyte percentage ($P<0.05$) and decreased in neutrophil percentage in G I comparing to pretreated period .The results also revealed non significant differences ($P>0.05$) in percentage of eosinophil and basophil along the different period of experiment . A significant increase in N/L index were shown after H_2O_2 treatment comparing to control ($P<0.05$), while levamizole treatment caused significant decrease in this index ($P<0.05$) in G I comparing to pretreated period.

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Table (1): Effect of 0.5% H_2O_2 and Levamizole on differential Leukocytes count (%)and Neutrophil/Lymphocytes index in male rabbits.

Treatment / Group		Control (C)	H_2O_2 treatment (GI)
Before treatment with Levamizole	Neutrophil/ Lymphocytes Index	0.73 ± 0.01 A a	0.95 ± 0.04 B a
	Lymphocytes	51.0 ± 0.7 A a	47.0 ± 0.5 B a
	Neutrophil	43.0 ± 0.6 a	45.0 ± 1.2 a
	Monocyte	4.6 ± 0.7 A a	5.0 ± 0.5 A a
	Basophil	1.0 ± 0.1	1.0 ± 0.1
	Eosiniphil	1.0 ± 0.2	1.6 ± 0.3
After Treatment with Levamizole	Neutrophil/ Lymphocytes Index	0.67 ± 0.03 A b	0.80 ± 0.03 B b
	Lymphocytes	54.0 ± 1.0 A b	51.0 ± 0.6 B b
	Neutrophil	37.0 ± 1.3 A b	38.0 ± 0.6 A b
	Monocyte	7.0 ± 0.5 b	8.0 ± 0.4 b
	Basophil	1.0 ± 0.1	1.0 ± 0.1
	Eosiniphil	1.0 ± 0.1	1.6 ± 0.2

Values expressed as means ± SE. n = 12/ group.
Capital letters denote between groups differences, P < 0.05 vs control
Small letters denote within groups differences, P < 0.05 vs control

Table (2) showed significant decrease (P<0.05) in total leukocyte count and platelets count following H2O2 intubation comparing to control group before treatment with levamizole. Meanwhile, treatment with levamizole cause significant increase in both parameters (p<0.05) comparing to pretreated period, however, levamizole treatment failed to normalized the values. The effect of H2O2 on percentage of active and total lymphocyte was observed in table (3). Significant suppression in these two parameters were observed in G I comparing to control before levamizole treatment. While , a significant elevation in active and total T-lymphocyte were observed in GI group due to levamizole treatment comparing to pretreatment period. Significant differences within group were exist (P<0.05).

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Table (2): Effect of 0.5% $H_2O_2$and Levamizole on total Leukocytes and platelets counts in male rabbits.

groups / treatment		Control (C)	H_2O_2treatment (GI)
Before treatment	Total WBC (x10^9 cell/L)	8.806 ± 0.332 A a	2.591 ± 0.121 B a
	platelets (x10^6 cell/L)	411.0 ± 20.0 A a	107.0 ± 18.0 B a
Treatment period	Total WBC (x10^9 cell/L)	12.315 ± 0.421 A b	5.812 ± 0.210 B b
	platelets (x10^6 cell/L)	521.0 ± 14.0 A b	298.0 ± 16.0 B b

Values expressed as means ± SE. n =12/ group,
Capital letters denote between groups differences, P < 0.05 vs control,
Small letters denote within groups differences, P < 0.05 vs control

Table (3): Effect of 0.5% H_2O_2 and Levamizole on percentage of active and total T-lymphocytes count in male rabbits .

groups / treatment		Control (C)	H_2O_2 treatment (GI)
Before treatment with Levamizole	Active T-lymphocyte %	30.0 ± 1.7 A a	10.0 ± 0.2 B a
	Total T-lymphocyte %	35.0 ± 1.9 A a	13.0 ± 0.4 B a
Treatment period	Active T-lymphocyte %	45.0 ± 0.5 A b	21.0 ± 0.4 B b
	Total T-lymphocyte %	47.0± 1.1 A b	25.0 ± 0.2 B b

Values expressed as means ± SE. n = 12/ group
Capital letters denote between groups differences, P < 0.05 vs control
Small letters denote within groups differences, P < 0.05 vs control

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Table (4) pointed to significant depression ($p<0.05$) in serum IgG concentration and phagocytic activity in H2O2 treated groups comparing to control before treatment with levamizole. While after levamizole treatment significant increase ($p<0.05$) in phagocytic activity were observed in GI comparing to pretreated period. However , levamizole treatment failed to correct IgG concentration in group I in spite of the non significant increase in IgG value in GI following levamizole treatment comparing to pretreatment period , its seems that levamizole failed to correct IgG value.

Table (4): Effect of 0.5% H_2O_2 and Levamizole on serum IgG(ng/ ml) concentration and phagocytic activity in male rabbits.

Groups / treatment		Control (C)	H_2O_2 treatment (GI)
Before treatment with Levamizole	IgG concentration	107.0 ± 1.0 A	13.0 ± 0.2 B
	Phagocytic activity %	12.0 ± 0.5 A a	2.0 ± 0.1 B a
Treatment period	IgG concentration	110.0 ± 1.2 A	17.0 ± 0.2 B
	Phagocytic activity %	19.0 ± 1.2 A b	8.9 ± 0.7 B b

Values expressed as means ± SE. n = 12 / group.
Capital letters denote between groups differences, P < 0.05 vs control
Small letters denote within groups differences, P < 0.05 vs control

DISCUSSION

In this investigation oral intubations of 0.5% of H2O2 to male rabbits caused immunosuppressant effects manifested by leucopenia, lymphocytopenia, thrombocytopenia, significant decrease in total and active lymphocyte percentage, reduction in phagocytic activity and serum IgG concentration with significant elevation in N/L index. The influence of exogenous exposure of animals to H2O2 (in vivo study) on immune system has not been well characterized. Few results were obtained from cell culture (14, 16, 17, and 32).

The role of ROS on lymphocyte proliferation is controversial , on one hand small amount (10 – 20 mM /L)of reactive oxygen intermediate (ROI) including H2O2 act as an important competence signals in T- lymphocyte including gene expression as well as cell proliferation when applied under reducing condition in vitro (33,34) , however , at high concentration (>20 Mm /L), it has inhibitory effect (35,36) where excess of

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ROI can damage DNA and cell membrane integrity (32,37) with subsequent depression in immune system (22,38,39,40) .

One possible explanation to the findings in this study (immunosuppressant effect of H_2O_2) may be attributed to oxidative stress produced duo to over production of ROS particularly hydroxyl radical (41,42,43,44,45) after H_2O_2 intubations .

It has been mentioned that exogenous exposure to high concentration of H_2O_2 cause oxidative stress in animals model (46,47) .Interestingly ,comparing to other cell type , immune cell may be at high risk of oxidative damage with an increased sensitivity to apoptosis and damaging cell membrane , where receptors for IL-Ks , hormones and IgG are placed leading impairment in crucial immune response (48,49).

Besides, high risk oxidative damage may be due to additional ROS generation through elevated NADPH oxidase activity (19,50) after H_2O_2 intubation. Meanwhile, hydrogen peroxide may stimulate macrophages for production of a variety of stress intracellular signaling pathway such as JNK and NF-KB leading to cellular damage (38,51). We can also estimated that the case of stress which may be induced by exposure to H_2O_2 may lead to over secretion of cortisol, the well- known immunosuppressant agent (52).

It has been mentioned that adequate vitamins E and C supplementation is necessary for maintaining perfect immune function , through stimulation of interleukin-2 production T-lymphocyte proliferation , depletion of platelets aggregation (53,54,55,56,57) .

Accordingly we can suppose that deficiency of these antioxidant vitamins may occur due to oxidative stress induced by H_2O_2 intubations in this study leading to depression in immune function index.

Concerning humeral immunity, in this study, H_2O_2 intubations caused significant depression in serum IgG concentration .Protein glycation due to exogenous H_2O_2 exposure may be responsible for such depression. The very aggressive hydroxyl radical which are produced from H_2O_2 can oxidize apolipoprotien and other plasma protein leading to suppression immunoglobulins including IgG (58, 59, 60) .Meanwhile, the SH group of protein can be attacked and undergo degredation by ROS leading to peroxidation of protein (61). Rendering protein highly susceptible to proteolysis (62), with subsequent reduction in plasma protein and IgG.

This investigation also pointed to the role of levamizole in ameliorating the immunosuppressant effect of H_2O_2. Levamizole has been found to be an immunostimulant both in experimental animals and in human being , and it has been used in immunosuppressant state (63,64).It has been mentioned that administration of levamizole increase guanidine monophosphate (GMP) levels both in monocyte and neutrophils and enhance hexose monophosphate shunt activity , therefore, stimulate phagocytosis and increase chemotactic response(65,66). Levamizole stimulate production of alpha & beta interferon and cytokine macrophages secretion (interleukin-1- and 6) which play an important role in T-lymphocyte function. Besides,it induced proliferation of these cells (monocyte and lymphocyte) in bone marrow (67) . Meanwhile, according to the ability of levamizole to boost depressant immune system and elevation the number of lymphocyte, subsequent depression in N/L index were observed in this study.

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