Effect of lipopolysaccharide on the liver function test

G. S. Abd and B. Hg. Abdul-Hameed
State company for veterinary services\Ministry of Agriculture

Abstract

Lipopolysaccharide (LPS; endotoxin), a major component of the outer membrane of the Gram-negative bacterial cell wall. Among the changes observed upon exposure to LPS are fever, circulatory shock, disseminated intravascular coagulation, and damage to numerous organs including the liver, acute lung injury and acute renal failure. 40 male Wister albino rats were divided in to 3 groups. Control group was given tap water during the experiment period. At the last day of the experiment, animal were injected intraperitoneally with distilled water. The second group was given ethyl alcohol with drinking water, at the end of the experiment 10 rats were injected intraperitoneally with distilled water and the other 10 rats were injected intraperitoneally with LPS (1µg/gm). Endotoxin group was given tap water during the experiment period, at the last day of the experiment period they were injected intraperitoneally with LPS (1µg/gm). for determining the biochemical tests the blood were collected in test tubes for obtaining the serum samples. Injection of LPS significantly increased the activity of alkaline phosphatase enzyme (SALP) and bilirubin level in serum as well as serum glutamic oxaloacetic transaminase (SAST) and glutamic pyruvic transaminase (SALT) were also increased compared with control rats. LPS significantly increased (SAST), (SALT), the activity of alkaline phosphatase enzyme (SALP) and bilirubin level in serum and it's regarded as a natural results of organs damage and dysfunction which caused by LPS insult.

تأثير متعدد السكريات الدهني على اختبار وظائف الكبد

غسان سامي عبد وبان غسان عبد الحميد
الشركة العامة للبيطرة/ وزارة الزراعة

الخلاصة

متعدد السكريات الدهني (الذيفان الداخلي) والذي يمثل الجزء الرئيسي في الغلاف الخارجي لبكتريا سالبة صبغة كرام. معظم التغيرات التي لوحظت هي ارتفاع درجة الحرارة، الصدمة الدموية. تجلط داخل الأوعية الدموية، أذى الأعضاء الداخلية والتي تشمل الكبد والأذى الحاد في الرئة والفشل الكلوي الحاد. 40 ذكر من جرذان نوع وستير البيضاء تم تقسيمها بصورة عشوائية إلى ثلاث مجاميع، تم إعطاء مجموعة السيطرة ماء مقطر خلال فترة التجربة. عند اليوم الأخير من التجربة تم حقن الحيوانات بالماء المقطر بالبريتون. المجموعة الثانية تم إعطائها الكحول الاثيلي مع ماء الشرب عند نهاية التجربة عشرة من الجرذان تم حقنها بالبريتون بالماء المقطر وعشرة أخرى حقنت بالذيفان الداخلي بجرعة مقدارها (1 ملغم/ غم) بالبريتون. مجموعة الذيفان الداخلي أعطيت ماء مقطر خلال التجربة في اليوم الأخير من التجربة حقنت بالذيفان الداخلي بجرعة (1 ملغم/ غم) بالبريتون لغرض تحديد التغيرات الكيموحيوية تم جمع الدم بواسطة أنابيب اختبار لتحليل عينات المصل. أظهرت النتائج ان حقن الذيفان الداخلي أدى إلى حدوث زيادة معنوية في مستوى الإنزيم القاعدي الفسنتيز (SALP) وزيادة في البليروليين في مصل الدم مع

زيادة نسبة (SAST) و(SALT) مقارنة مع مجموعة السيطرة. من ذلك نستنتج بوجود زيادة معنوية في كـل مـن إنزيمي (SAST) و(SALT) وزيادة فعالية إنزيم (SALP) والبليروليبين في مصل الدم ويرجع سبب ذلك كنتيجـة طبيعية للأذى الحاصل وعدم اختلال وظيفة الكبد بسبب الذيفان الداخلي.

Introduction

Endotoxins, also called lipopolysaccharides (LPS), are a major component of the outer membrane of Gram-negative bacteria. They are composed of a hydrophilic polysaccharide moiety, which is covalently linked to a hydrophobic lipid moiety (Lipid A) (1, 2, 3). LPS from most species is composed of three distinct regions: the O-antigen region, a core oligosaccharide and Lipid A (Lip A). The lipid A is the most conserved part of endotoxin and is responsible for most of the biological activities of endotoxin, i.e. its toxicity (4). The O-antigen is generally composed of a sequence of identical oligosaccharides (with three to eight monosaccharides each), which are strain specific and determinative for the serological identity of the respective bacterium (5). Lipopolysaccharide (LPS; endotoxin) is among the most potent modulators of the innate immune system. It is responsible for a host of toxic effects that occur in patients infected with Gram-negative bacteria, including fever, disseminated intravascular coagulation, and hemodynamic changes, which may lead to multiple organ failure characteristics of the septic shock syndrome (6). Lipopolysaccharide triggers increased production of avariety of proinflammatory mediators, such as tumour necrosis factor-α (TNF-α), interleukin 1β (IL-1β), IL-6, and nitric oxide (NO) (7), as well as increased production of chemokines and cell adhesion molecules, leading to the recruitment of several lineages of white blood cells, including neutrophils, to the liver (8)**.** If uncontrolled, these proinflammatory responses contribute to liver injury and acute hepatic failure (9, 10, 11). Disseminated intravascular coagulation (DIC) is characterized by a systemic activation of the blood coagulation system, which results in the generation and deposition of fibrin, leading to microvascular thrombi in various organs and contributing to the development of multiorgan failure (12). Consumption and subsequent exhaustion of coagulation proteins and platelets, due to the ongoing activation of the coagulation system, may induce severe bleeding complications, although microclot formation may occur in the absence of severe clotting factor depletion and bleeding (13). Endotoxin causes multiple organ dysfunctions, including acute lung injury. Acute lung injury is characterized by acute lung inflammation involving the local recruitment and activation of polymorphonuclear neutrophils1 and the release of proinflammatory mediators, (14, 15) proteases, and reactive oxygen and nitrogen species. Eventually, these processes can cause alveolar-capillary damage with high permeability pulmonary edema and alteration of lung mechanics, resulting in severe gas exchange abnormalities (16). Sepsis is a common cause of acute renal failure (ARF) (17, 18), an entity in which the abrupt decline in glomerular filtration rate is associated with the pathological lesion of acute tubular necrosis (18).

Materials and Methods

- **Treatment of animals:**

40 male Wister albino rats were housed in cages (5 rats per cage) and randomly divided in to 3 groups.

1. Control group: 10 rats were given tap water during the experiment period (90 days). At the end of 90 days the animal were injected intraperitoneally with distilled water.
2. The second group: (20 rats) were given ethyl alcohol with drinking water and the dose of ethanol was increased progressively till it concentration reached 20% at the 5th week,

at the end of the experiment 10 rats were injected intraperitoneally with distilled water and the other 10 rats were injected intraperitoneally with LPS (1µg/gm) (LPS from segma com).

3. Endotoxin group: 10 rats were given tap water during the experiment period, at the last day of the experiment period they were injected intraperitoneally with LPS (1µg/gm) (LPS from segma com).

Twenty– four hours after experimental period the animals were killed and the blood were collected in test tubes for obtaining the serum samples to determine the biochemical tests.

Results

Injection of LPS significantly increased the activity of alkaline phosphatase enzyme (SALP) (Table 1) compared with control rats. (Table 2) shows that there is significant increase in bilirubin level in serum compared with control rats. Furthermore plasma biochemistry glutamic pyruvic transaminase (SALT) and glutamic oxaloacetic transaminase (SAST) were also increased compared with control rats (Table 3,4).

Table (1) Effect of endotoxin to the activity of alkaline phosphatase enzyme (SALP) (IU/L)

	Control	Endotoxin
1	89	138
2	110	121
3	113	149
4	107	82
5	121	131
6	75	89
7	50	92
8	71	82
9	71	92
10	60	107

(P< 0.1)

Table (2) Effect of endotoxin on the level of serum total bilirubin (mg/100 ml)

	Control	Endotoxin
1	0	0.3
2	0	0.2
3	0	0.5
4	0	0.2
5	0	0.5
6	0	0.1
7	0	0.2
8	0	0.3
9	0	0.2
10	0	0.1

(P< 0.1)

Table (3) Effect of endotoxin on serum (SALT) level (IU/L)

	Control	Endotoxin
1	12	18
2	18	18
3	18	29
4	20	22
5	24	18
6	9	24
7	16	22
8	16	22
9	16	36
10	12	24

(P< 0.1)

Table (4) Effect of endotoxin on serum (SAST) level (IU/L)

	Control	Endotoxin
1	64	96
2	60	80
3	60	96
4	47	72
5	80	88
6	31	37
7	31	47
8	39	60
9	35	41
10	17	51

(P< 0.1)

Discussion

Our results show that there is significant increase in alkaline phosphatase enzyme (SALP) activity. Previous studies show that increased alkaline phosphatase level are associated with hepatic damage and it has been demonstrated that alkaline phosphatase attenuates the lipopolysaccharide mediated inflammatory response because alkaline phosphatase plays essential roles in detoxification of pathogenic bacterial (LPS) endotoxin by phosphorylation it's lipid A moiety (19, 20, 21). We show that there is significant increase in bilirubin level in serum compared with control rats. This mean that the liver is incapable of removing bilirubin from the blood (22). Plasma bilirubin increased due to hepatocyte dysfunction as a result of hepatocellular disease (eg. Infective, auto immune, toxic, septic, hypoxia) (23, 24) demonstrated that hyper bilirubinemia can occur after (G+ve) or (G -ve) bacteremia and endotoxin– mediated cytokine release is the likely cause of septic jaundice .a variety of bacterial infection affect the liver either as a result of direct hepatocellular or biliary invasion or through the production of toxins (LPS) or endotoxin is a potent inducer of cytokines. endotoxin– mediated cytokine release may be the bases for jaundice of many disorders, including sepsis. Early studies showed areduction in bile flow and biliary excretion after administration of endotoxin to isolate perfused rat liver. Studies have shown that canalicular bile acid and organic anion transport are markedly impaired in endotoximia therefore, endotoximia severely impaires the transport of organic anions at both the sinusoidal and canalicular membrane. Because of impaired hepatic organic anion transport both bile acid-dependent and bile acid-independent component of bile flow decreased with administration of endotoxin. LPS significantly increased blood (SALT) and (SAST) (markers of organs injury) (25). (SALT) and (SAST) is acytoplasmic

hepatocellular enzyme whose increase in blood is highly indicative for hepatocellular damage or injury from different types of disease (26). Endotoxin shock can induce the production of nitric oxide (27) and several inflammatory mediators such as TNF-α,IL-6 and IL-1β leading to multiple organ dysfunction and death (27, 28).

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