CORRELATION BETWEEN PLASMA ENDOTHELIN-1, BLOOD GLUCOSE AND SERUM CALCIUM IN MALE RATS INSTILLATED WITH BLEOMYCIN

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ABSTRACT

Endothelin-1 is profibrotic by stimulating fibroblast replication, migration, contraction, collagen synthesis and secretion while decreasing collagen degradation.In the present study,20male rats weighing 240 grams and 10-12 weeks old were utilized.Pulmonary fibrosis was induced by a single instillation of bleomycin (90 mg/kg).After 7 days of instillation, elevated plasma ET-1 was considered as bleomycin induced pulmonary fibrosis. High dose of bleomycin instillation caused significant increase in plasma ET-1as compared with normal saline instillation. Blood glucose and serum calcium were also markedly elevated. Statistical analysis using Pearson coefficient of correlation revealed that there was a positive relationship between plasma ET-1 and blood sugar (r=0.7). The same correlation was detected between plasma ET-1 and serum calcium (r=0.7).The present study concluded thatthere is a strong correlation between plasma ET-1 and blood glucose and it may be due to decreaseof insulinsensitivity, and inhibits β-cells to release insulin .In addition,the role of calcium ions cannot be excluded in raising blood glucose by ET-1.

Keywords:Endothelin-1, Bleomycin, blood glucos , calcium ions

INTRODUCTION

In 1988, Yanagisawa and coworkers purified a 21- amino acid peptide from porcine aortic endothelial cells that is a powerful constrictor. The discovery of ET-1 and peptide endothelin-2 (ET-2) and endithelin-3 (ET-3) has stimulated considerable interest. Instillation of intratrachealbleomycin is a frequently used animal model of lung injury and fibrosis. The model is characterized by an initial alveolitis consisting mainly of neutrophils infiltration with pathologic changes of diffuse alveolar damage with peak injury around the seventh day. The inflammatory phase remits, and collagen production gradually increased to maximal levels at days 21 to 30 (Teder and Noble, 2000).

Interstitial pulmonary fibrosis is a consequence of many types of severe or sustained lung inflammation. Bleomycin is a mixture of glycopeptides derived from *Streptomyces verticillus*, is a potent chemotherapeutic agent and is known to produce pulmonary fibrosis in humans as well as in experimental animals (Wang *et al*., 2002). Furthermore, Serrano-Mollar*et al.,* (2002) showed that bleomycin-induced lung fibrosis appears to be the consequence of a primary inflammatory lesion characterized by an accumulation of alveolar macrophages and neutrophils in the lower respiratory tract. In addition, reactive oxygen species generated from neutrophils can induce lung injury and fibrosis, Park et al.,(1997)

Endothelin-1 is profibrotic by stimulating fibroblast replication, migration, contraction, collagen synthesis and secretion while decreasing collagen degradation. Thus blocking ET-1 might be beneficial in reducing scar formation in pulmonary fibrosis (Shi-Wen *et al*., 2004). Endothelin-1 additionally enhances the conversion of fibroblast into contractile myelofibroblast (Villaschi and Nicosia, 1994; Sun *et al*., 1997). Increased in ET-1 precedes the development of pulmonary fibrosis (Fagan *et al*., 2001).Mutsaers *et al*., (1998) demonstrated that elevation of ET-1 levels prior to an increase in collagen content, along with its localization within developing fibrotic lesions, provides further evidence of profibrotic role for ET-1 in the pathogenesis of pulmonary fibrosis.Park *et al*.,(1997) also demonstrated that ET-1 is involved in the pathogenesis of bleomycin-induced pulmonary fibrosis in the rodent model and that blockage of its receptors reduces the fibrosis.

In summary, circumstantial evidence for a role of ET-1 in lung fibrosis has accumulated, but whether this is a causative or bystander role remains unknown (Teder and Noble, 2000).The main purpose of the present study was to find out the correlation between the elevated plasma ET-1 in bleomycin instillation and blood glucose concentration .Such

correlation, if present , may demonstrate how pulmonary disorders caused by bleomycin increase blood sugar ,the most common symptoms of diabetes mellitus

MATERIALS AND METHODS

Animals and housing

Adult male albino rats (*Rattus norvegicus*) bred in the animal house of Biology Dept. /College of Science/University of Salahaddin were used for the present study. In this study, 20 healthy rats weighing 240 grams and 10-12 weeks old were used in this study. Animals were housed under standard laboratory conditions (12 h light: 12 h dark photoperiod, 22 ± 2 Cº, and fed on standard rat pellets and tap water *ad libitum*.

The study was performed on ten adult male rats. (pretreatment body weight 200 to 260 g).They were anesthetized with 100mg/kg of ketamin (Hikma pharm ceatical, Amman. Jordan) injection (Keane *et al*., 1999).Bleomycin (Kayaku, Tokyo. Japan) solution was prepared immediately before administration as a single dose directly into the trachea. Using aseptic techniques, a single incision was made at the neck and the muscle covering the trachea was snipped to expose the tracheal rings. The rats then injected with 90 mg / kg of bleomycin in 0.3 ml of sterile 0.9% sodium chloride through a 27 gauge needle. The controlled animals were given an equal volume of sterile 0.9% saline only. After bleomycin instillation, the incisions were sutured by surgical thread (size 0/3, cutting needle 22mm). (Ebihara*et al*., 2000; Hamaguchi*et al*., 2002).

Experimental design

This experiment was designed as the following:

Group1: Normal saline instillation.(Control)

Group2: Bleomycin (90mg / kg) instillation.

After 7 days all the rats were anesthetized with ketamine (100mg / kg). And sera were preserved at-85 Cº until their use. This experiment was repeated twice to confirm the results

Endothelin-1-hormone immunoassey

Plasma ET-1 was measured by enzyme linked immunosorbent assay (ELISA)(Bio-tek , USA) (Rossi *et al* ,2000)

Blood glucose determination

Control and experimental rats were fasted overnight for 12 hours but provided with water *ad libitum*. Blood samples from the rats were collected by heart puncture method and analyzed for glucose level employing glucosticks with the glucometer. (Accu-Chek, Roch diagnostic GmbH, Mannheim, Germany)(Syiem *et al* ,2002).

SERUM TOTAL CALCIUM

Spectrophotometric method was used for serum calcium determination. Arsenazo III reacts with calcium in a slightly acid medium to form blue - purple complex. The intensity of the color is proportional to the calcium concentration. SYRBIO diagnostic reagents for laboratories under license of EUROBIO laboratories Paris – FRANCE use for determination serum calciumand the results were expressed as mg/dl. (Chawla, 2003)

STATISTICAL ANALYSIS

Statistical analysis was performed by using SPSS (Version 11.5). Results are expressed as mean ± S.E. The correlation analysis was done with Parsons test. Independent T-test was also used.p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Bleomycin caused a marked increase in plasma ET-1 ($p<0.001$)(Figure 1) as compared with control The mechanism by which bleomycin increases plasma ET-1 is not fully understood. However, several reports indicated that ET-1 is strongly related with pulmonary fibrosis produced by bleomycin (Villaschi and Nicosia, 1994; Park *et al.*, 1997: Mutsaers*et al.*, 1998; Shi-Wen *et al.*, 2004).

Blood glucose and serum calcium were also markedly elevated in bleomycin treated rats (Figure 2 and3).Using Pearson correlation ,tatistical analysis revealed that there was a positive relationship between plasma ET-1 and blood sugar ($r=0.7$). The same correlation was detected between plasma ET-1 and serum calcium ($r=0.7$), (Table 1). Recently, there has been known that elevated ET-1 levels may cause insulin resistance in certain pathophysiological states and because blood flow to skeletal muscle tissues is an additional determinant of skeletal muscle glucose uptake, the possibility can be raised that chronic ET-1 administration reduced skeletal muscle blood flow and this could contribute to the insulin flow and insulin resistance (Wilkes *et al.*, 2003). Furthermore, it has been shown that insulin can promote ET-1 gene expression

(Ferri*et al.*, 1995). Thus, one possibility is that insulin resistance leads to hyperinsulinemia, which causes increased ET-1 levels, which then further exacerbates the insulin-resistance state. In this way, a positive feedback system may exist *invivo*, in which insulin resistance begets more insulin resistance through the ET-1.In addition,there is a possibility that ET-1 can inhibit pancreatic β-cell function (Teuscher*et al.*, 1998).In conclusion,the present study suggested that there is a strong correlation between plasma ET-1 and blood glucose and it may be due to decrease of insulin sensitivity, and inhibits β-cells to release insulin .In addition, the role of calcium ions cannot be excluded in raising blood glucose by ET-1.

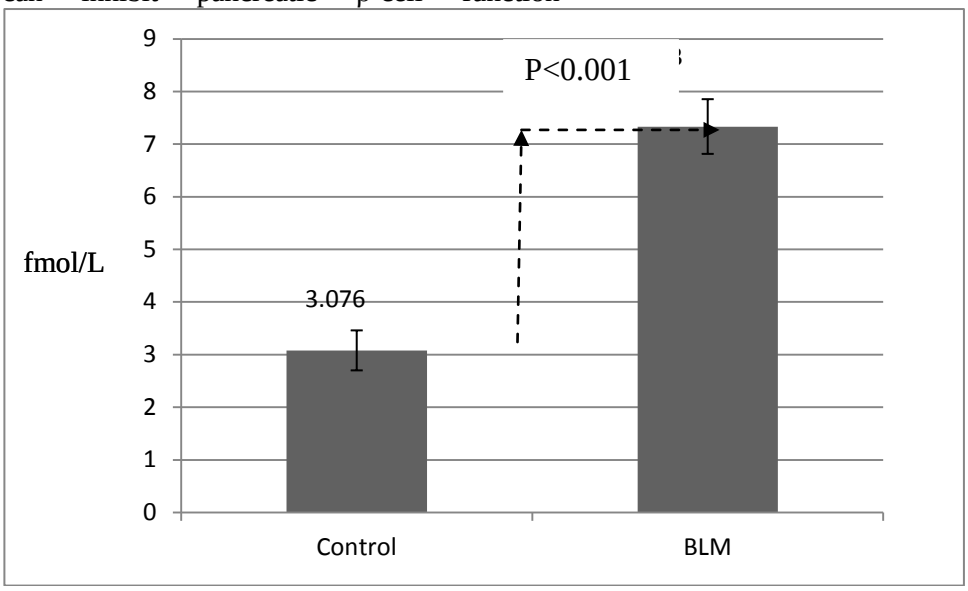

Figure (1):Effect of instillation of bleomycin on plasma ET-1 in male rats.

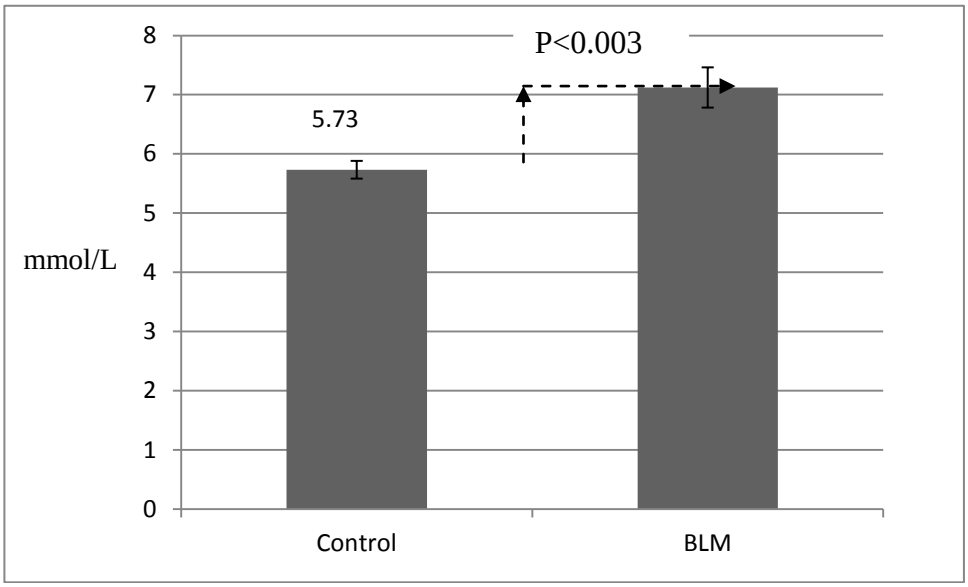

Figure (2): Effect of instillation of bleomycin on blood glucose in male rats.

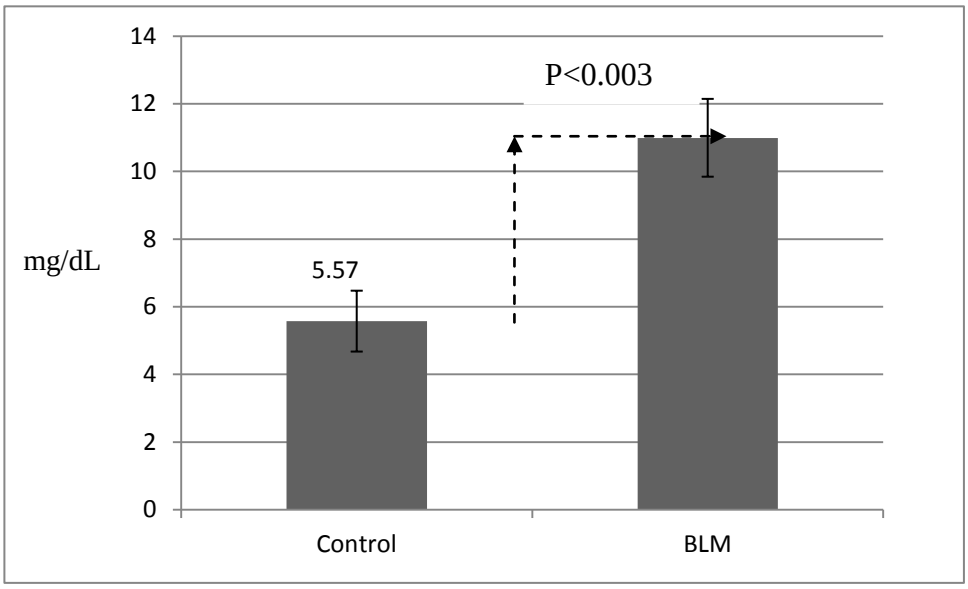

Figure (3): Effect of instillation of bleomycin on serum total calcium in male rats.

Table 1:Correlations between plasms ET-1,blood glucose and serum calcium in male rats instillated with normal saline and bleomycin

Correlated parameters	Pearson correlation r- values		Statistical decision
Plasms ET-1 and blood glucose	n1=10,n2=10	0.71	p<0.021
PlasmaET-1 and serum calcium	n1=10,n2=10	0.78	p<0.008

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پەیوەندی نێوان ئیندوپیلین-1 لە پلاناریا و شەکری خوێن و کالیسیۆم لەزەرداوی خوێندا لە جورجی پێدراو بەماددەی بلیۆمایسین

پوختە

ئیندوپیلین-1 کەبە دروستکەری ریشالبوونی سیەکان دادەنرێت لەڕێگەی هاندانی فایبرۆبلاست و گرژبوون و ڕۆیشتن و بەرهەم هێنانی کۆلاجین و فرێدانی وە کەمکردنەوەی کرداری هەڵوەشانەوی کۆلاجین. لەم لێکۆڵینەوەیەدا 20 جورجی سپی 240 گم و 10-12هەفتە لەتەمەنیان بەکارهێنران. بەڕیشاڵ بوونی سی یەکان بەهۆی دانی بلیۆمایسین بەڕێژەی (90ملگم/ کگم کێشی لەش) دروست کران. ڕێژەی ئیندوپیلین-1 بەرزبۆوە. بەپێدانی جورجەکان بەماددەی بلیۆمایسین بۆماوەی 7ڕۆژ بەبەراورد لەگەڵ جورجی کۆنترۆڵ پێدراو بە خوێی ئایزۆتۆنی هەروەها ڕێژەی شەکر و کالیسیۆم بەرزبوونەوە. شیکاری ژمێریاری دەریانخست کە پەیوەندیەکی بەهێز لەنێوان ئیندوپیلین-1 و شەکری خوێندا هەیە. هەروەها ئەم پەیوەندیە لەگەڵ ڕێژەی کالیسیۆمیش هەیە . بەرەنجامەکان پێشنیار دەکەن کە پەیوەندیەکی بەهێز لەنێوان پلازمای ئیندوپیلین-1 وە شەکری خوێن هەیە، ئەمەش لەوانەیە بۆ ئەوە بگەڕێتەوە کە ئیندوپیلین-1 کاریگەری هۆرمۆنی ئەنسۆلین بۆ ڕیسێپتەرەکانی کەم بکاتەوەوخانەکانی بێتا لە پەنکریاس ڕابگرێت لەفرێدانی ئەنسۆلین. ئایۆنی کالیسیۆمیش ڕۆڵی هەیە لەبەرزکردنەوەی ڕێژەی شەکری خوێن.

علاقة الاندوثيلين–1 البلازمي و الكلوكوز الدمي والكالسيوم المصلي في ذكور الجرذان المعاملة بمادة البليومايسين

الخلاصة

يعتبر الاندوثيلين–1 كمحضز لتليف الرئوي عن طريق تحفيز الفايبروبلاست و هجرهو تقلصه و انتاج الكولاجين وافرازه وانخفاض عمليه هدم الكولاجين. في هذه الدراسة، استخدمت 20 من الذكور الجرذان بوزن 240 غم و 10–12 اسابيع من العمر. استحدثت عمليه التليلف الرئوى باستخدام جرعة واحدة من البليومايسين بتركيز (90ملغم/ كغم وزن الجسم). بعد 7 أيام من المعاملة ، ارتفعت تركيز الاندوثيلين–1 البلازمي قارنه بمجموعة السطيرة والمعاملة بملح الايزوتوني. ان تركيز الكلوكوز في الدم و تركيز الكالسيوم في المصل ايضاً ازدات بشكل المعنوي. اظهرت التحليل الاحصائي مستخدماً المعامل الارتباطي بان هنالك علاقة طَردية بين الاندوثيلين–1 البلازمي و تركيز الكلوكوز و تركيز الكاليسيوم . تسنتتج من الدراسة الحالية بان هناك علاقة قوية بين الاندوثيلين–1 البلازمي و تركيز الكلوكوز وقد يعود السبب الى انخفاض في حساسية الانسولين لمستقبلاته البيتا لافراز هرمون الانسولين اضافة الى ذلك لايمكن ابعاد دور الكالسيوم في ارتفاع الكلوكوز بسبب اعطاء مادة البليومايسين