The Possible Protective Effect of Vitamin E on Pancreas of Adult Male Albino Rats Treated with Bleomycin Sulfate

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Abstract

Background: Bleomycin sulfate is a chemotherapeutic antibiotic that may have adverse effects on both the exocrine and endocrine component of the pancreas.

Aim of Study: This study was designed to assess the effect of bleomycin sulfate on pancreas of adult male albino rats and the possible protection by vitamin E administration.

Material and Methods: Twenty-five adult male albino rats were divided into three groups: Group I: Control group, Group II: Bleomycin sulfate treated group and Group III: Bleomycin sulfate and vitamin E treated group. At the end of the experiment, pancreas was taken out and processed for light, electron microscopic examinations and anti-insulin immunohistochemical staining. The percentage area of anti-insulin antibody reaction was measured and statistically analyzed.

Results: Treatment with bleomycin sulfate showed variable marked histopathological changes. Distortion of the normal architecture of the pancreatic tissue with areas of hemorrhage, vacuolation and necrosis either in the acini or islets of Langerhans with loss of differentiation between beta cells and alpha cells. Histopathological changes were diminished after receiving vitamin E. The acinar cells were more regular with basophilic rounded basal nuclei and apical acidophilic cytoplasm. The islet of Langerhans restored its normal shape and size with nearly regular out line.

Conclusion: Vitamin E could protect the pancreas against bleomycin sulfate-induced alteration in adult male albino rats.

Key Words: Bleomycin sulfate – Oxidative stress – Pancreas – Vitamin E.

Introduction

In patient who have palliative treatment, bleomycin sulfate is used either as a single agent or in combination as in Hodgkin, non-Hodgkin lymphomas, squamous cell carcinoma, malignant pleural effusion, and testicular carcinoma [1]. Bleomycin treatment causes development of a pre-diabetic pattern to pancreas whose degree of incidence of reversibility is still unknown [2]. Vitamin E (α-Tocopherol) is one of the main lipid soluble antioxidant vitamins. It is present in membranes and lipoproteins, so it is regarded as membrane antioxidant. It stops the chain reaction of lipid peroxidation by scavenging intermediate peroxyl radicals [3]. Vitamin E supplementation may protect the liver and kidney and other organs against environmental pollutants such as ozone, chemotherapy and radiotherapy in a dose dependent manner as mentioned by [4]. Vitamin E has a role in neurological functions and inhibition of platelet aggregation and prevention of oxidation of polyunsaturated fatty acids [5].

Material and Methods

1- Animals:

This study was conducted on 25 adult male albino rats (of average weight 200gm of each rat). The animals were kept in a standard diet and water. They were kept in clean plastic cages, five rats/cage under standard laboratory and environmental conditions in the period from January 2015-January 2016. This study was conducted in accordance with ethical procedures and policies approved by Animal Care Committee of Faculty of Medicine, Al-Azhar University, Cairo.

2- Drugs:

Vitamin E was purchased as a gelatinous capsule from Pharco Pharmaceutical Company Cairo, Egypt. Each capsule contained 400mg vitamin E which dissolved in oily solvent (corn oil). Bleomycin sulfate was purchased as vial with a trade name (Blenoxane) from Bristol-Myers Squibb Company Cairo, Egypt.
Experimental design:

Animals were divided into three groups:

Group 1: Control group contained 15 rats which is subdivided into three equal subgroups, each subgroup contained (5) rats:
- Group 1a: (−ve control group): Each rat received no medications.
- Group 1b: (+ve control group): Each rat received only a single daily dose of 0.7ml of oily solvent orally for 10 days and served as a positive control.
- Group 1c: (Vitamin E group): Each rat received Vitamin E at a single daily dose of 400mg/kg body weight orally by a gastric tube for 10 days. This dose was equivalent to average human therapeutic dose [6]. Rat dose was calculated according to the formula adapted by [7].

Group II: (Bleomycin sulfate treated group): Contained 5 rats, each rat received bleomycin sulfate at a single daily dose of (2.7mg/kg/day) body weight by intramuscular injection for 10 days. This dose was equivalent to average human therapeutic dose [8]. Rat dose was calculated according to [7].

Group III: (Bleomycin sulfate and Vitamin E treated group): Contained 5 rats, each rat received both bleomycin sulfate as in group II and Vitamin E as in Group 1c daily for 10 days. Vitamin E was administered one hour prior to treatment with bleomycin sulfate [9].

By the end of the experiment the rats of all groups were anesthetized by ether, sacrificed by decapitation and the pancreas removed carefully. The specimens of the pancreas were obtained from the tail of the pancreas for light, electron microscopic examination and anti-insulin immunohistochemical staining.

Histopathological preparation:

1- Preparation for light microscopic examination:
Specimens were immediately fixed in 10% neutral buffered formaldehyde and processed for paraffin sections of 5 micrometers thickness. Sections were stained with Hematoxylin and Eosin (H & E) stain and Masson's Trichrome (MTC) stain [10].

2- Preparation for Anti-insulin immunohistochemical staining: This was performed on 5 micrometers thick paraffin sections. The tissue sections were deparaffinized in xylene, immersed in 3 percent hydrogen peroxide. The tissue sections were incubated with avidin-biotin peroxidase system. The primary antibodies used were mouse monoclonal insulin antibodies (Medico Company, Egypt) at a dilution of 1:100 that incubated with slides for 1h at room temperature. Then, the sections were counter stained with Meyer's hematoxylin [11].

Morphometric study:
The image analyzer was used to measure the area percent of staining of immunoreactive B-cells in the islets of Langerhans. Selected areas could be measured by the computer system. Results were expressed as area percent. The mean ± SD was calculated from the measured area percent of 5 different X100 high-power fields. Morphometric measurements were performed by using software Leica Qwin500 (Leica Microsystems, Switzerland) at Pathology Department, Faculty of Dentistry, Cairo University.

Statistical analysis:
The results were collected, charted, statistically analyzed and represented graphically. Values were presented as mean, Standard Deviation (SD) and confidence intervals values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data), therefore one-way analysis of variance (ANOVA) and Tukey’s post hoc tests were used for comparison. The significance level was set at \( p \leq 0.05 \). Statistical analysis was performed with SPSS 18.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

3- Preparation for electron microscopic examination: Specimens fixed in glutaraldehyde for 18-24h then washed in phosphate buffer (pH 7.4) and post-fixed in isotonic 1% osmium tetroxide then processed. Semi thin sections were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate [12] examined and photographed with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Results

Histological results:
The results of control subgroups (group Ia, Ib and Ic) were similar so results of these groups were pooled as one control group (Group I).

1- Light microscopic findings:

Group I: Light microscopic examination of H & E stained pancreatic sections of Group I revealed the normal pancreatic lobules packed with serous
acini represented the exocrine portion of the pancreas. Each serous acinus was lined by pyramidal acinar cells arranged around narrow acinar lumen. The acinar cells had basophilic rounded basal nuclei and apical acidophilic cytoplasm, which was occupied by acidophilic zymogen granules. Some acinar cells were binucleated. The pancreatic islets of Langerhans which represented the endocrine portion appeared as a well circumscribed, pale stained oval areas inside the pancreatic lobules. They were formed of groups of cells arranged in irregular, branching and anastomosing cords separated by blood capillaries. The beta (β-cells) occupied most of the islet core, while alpha (α-cells) was more numerous at the islet periphery Fig. (1).

Group II: Showed complete alteration of the pancreatic tissue with areas of hemorrhage and necrosis. The exocrine pancreas showed the acinar cells with cytoplasmic vacuolations with pyknotic nuclei. Islet of Langerhans appeared shrunken, vacuolated, distorted even that β-cells cannot be identified from the α-cells Fig. (2).

Group III: Showed marked signs of improvement with restoration of the pancreatic parenchyma. The acinar cells were more regular with basophilic rounded basal nuclei and apical acidophilic cytoplasm. The islet of Langerhans restored its normal shape and size with nearly regular outline and appeared in close associations with the ducts. The α- and β-cells of islet can be identified from each other. The blood vessels and the ducts appeared not dilated or congested and nearly normal Fig. (3).

Sections of the pancreas stained with Masson’s Trichrome Stain (MTC) from the control group showed fine collagen fibers distribution within the parenchyma of the pancreas, pancreatic acini, islets of Langerhans, and pancreatic ducts as well as in the blood vessels wall Fig. (4). In Group II MTC stained pancreatic sections showed marked increase in the collagen fibers deposition within the parenchyma of the pancreas. Blood vessels and the ducts were slightly dilated with increase in the collagen fibers in their walls Fig. (5). In group III MTC stained sections nearly normal collagen fibers distribution in the blood vessel wall. The ducts were slightly dilated with scanty connective tissue fibers in its walls. The islet of Langerhans appeared normal in shape and size with scanty connective tissue fibers within it Fig. (6).

Anti-insulin immunohistochemical findings:

Immunohistochemical results revealed that, the β-cells of the islets of Langerhans in group I stained by strong positive reaction for anti-insulin antibodies in the form of dark brown cytoplasmic granules Fig. (7). In contrast in group II β-cells revealed weak reaction in some islets in the same sections Fig. (8). In Group III, β-cells were stained by strong positive reaction similar to the control Fig. (9).

Statistical results:

The highest mean area percent of anti-insulin immunostaining was recorded in group I while there was a significant decrease in the mean area percentage was recorded in group II. The administration of Vitamin E (Group III) caused a significant increase in the mean area percentage of anti-insulin immunostaining as compared with group II (Table 1).

Electron microscopic results:

Ultra-structural examination of Group I showed the cytoplasm of the acinar cells which was characterized by the presence of large number of electron dense zymogen granules. The content of the zymogen granules appeared with variable densities representing formative stages of the granules (the zymogen granules and the pro-zymogen granules). Regularly arranged rough endoplasmic reticulum (r E R), normal mitochondria and lysosomes. The nuclei of the acinar cells, nuclear membrane (nuclear envelope), the nucleolus and the chromatin matrix showed regular pattern Fig. (10).

In Group II, showed many disturbed structures of acinar cells. The cytoplasm of acinar cells showed decreased number and density of zymogen granules, swollen rough endoplasmic reticulum, ballooning with ill-defined cristae of the mitochondria. Nuclei of acinar cell show dilatation, folding and indentation of the nuclear membrane with irregularity in its outline Fig. (11).

Group III showed marked pancreatic improvement. The acinar cell showed nearly normal zymogen granules both in amount & in density with normal mitochondria in shape and size. The nucleus looks nearly normal with regular nuclear membrane Fig. (12).

Electron microscopic examination of the islets of Langerhans cells in group I which contained many β-cells and many α-cells. The islet cells were characterized by the endocrine secretory granules. The cytoplasm of the β-cells contains secretory granules which were pleomorphic with rounded shape, different sizes and contain homogenous cores, which were separated from the surrounding membrane by wide electron-lucent haloes. Some
granules had an electron dense core and other had mild or moderate dense core. These granules contained insulin. The nuclei of β-cells were regular in outline and contain nucleolus moreover, hetero and euchromatin. Heterochromatin were attached to the nuclear envelop while the rest of the nucleus contain mostly euchromatin Fig. (13).

In group II, the β-cells showed multiple signs of degeneration, as marked degranulation of the β-cell secretory granules in the form of decrease in amount and decrease in density, furthermore some granules appeared degranulated, empty or vacuolated. The nucleus also showed disturbance in the chromatin matrix Fig. (14).

In Group III, the β-cells showed marked improvement with increase in its secretory granules which appeared as the control group, which was rounded in shape, variable in size and contained homogenous cores which were separated from surrounding membrane by wide electron-lucent haloes. Nucleus was regenerated again to the nearly normal shape and size Fig. (15).

In Group I, the α-cells granules were smaller in size than those of β-cells granules, also, the granules of α-cells were more electron dense core (darker)and surrounded by narrower haloes as compared with the granules of β-cells. Also, the nuclei of the α-cells were regular in shape (rounded or oval) but smaller than those of β-cells Fig. (16).

In group II, the α-cells appeared affected with nuclear degeneration and few secretory granules around rough endoplasmic reticulum (rER) Fig. (17).

In group III, the α-cells showed marked improvement with marked improvement with regeneration...
Fig. (5): A photomicrograph of transverse section of group II shows increase in collagen fibers distribution around the acini (a), islet of Langerhans (i), wall of dilated blood vessels (v) and duct (D) (MTC X100).

Fig. (6): A photomicrograph of transverse section of the pancreas of group III shows nearly normal collagen fibers deposition in islet of Langerhans (i), wall of blood vessel (V) and slightly dilated duct (D) (MTC X100).

Fig. (7): A photomicrograph of transverse section of anti-insulin immune-reactivity of the pancreas of group I shows strong positive immune-reaction as dark, brown-stained pancreatic B-cells with strong positive immune-reaction in the islets of Langerhans (arrows) (Anti-insulin Immunostaining X100).

Fig. (8): A photomicrograph of transverse section of anti-insulin immune-reactivity of the pancreas of group II shows weak reaction in some islets (arrows) (Anti-insulin Immunostaining X100).

Fig. (9): A photomicrograph of transverse section of anti-insulin immune-reactivity of the pancreas of group III shows strong positive immune-reaction similar to the control (arrows) (Anti-insulin Immunostaining X100).

Fig. (10): Electron micrograph of the pancreas of group I shows the acinar cell with regular arranged rough endoplasmic reticulum (rER) around the nucleus (N), regular appearance of mitochondria (MC) and zymogen granules (ZG) (EM Mag. X12000).
Fig. (11): Electron micrograph of group II shows the acinar cell with swollen mitochondria (MC), dilatation of the rough endoplasmic reticulum (rER), decrease zymogen granules (ZG) and irregular nucleus (N) (EM Mag. X12000).

Fig. (12): Electron micrograph of group III shows the acinar cell with nearly normal zymogen granules (ZG), nucleus (N) and mitochondria (MC) (EM Mag. X12000).

Fig. (13): Electron micrograph of Group I shows β-cell with cytoplasmic granules (B-Gr) normal nucleus (N) and prominent nucleolus (Nu) (EM Mag. X12000).

Fig. (14): Electron micrograph of Group II shows β-cell with degranulation its secretory granules (B-Gr) and nucleus (N) (EM Mag. X12000).

Fig. (15): Electron micrograph of Group III shows marked improvement in β-cell secretory granules (B-Gr) and nucleus (N) (EM Mag. X12000).

Fig. (16): Electron micrograph of the pancreatic islet of Group I shows β-cells with their granules (A-Gr). Notice blood capillary (bl-cap) (EM Mag.)
The present study clarified that pancreatic structure was affected markedly by bleomycin sulfate which exerted deleterious histopathological effects on pancreas of adult male albino rat. Distortion of the normal architecture of the pancreatic tissue with areas of hemorrhage, vacuolation and necrosis either in the acini or islets of Langerhans with loss of differentiation between α & β cells. These findings were in line with the work of [13] who found abnormality in the cycle of zymogen enzymes secretion, which might lead to the formation of intra-acinar vacuoles. They attributed that vacuolation might be due to the toxic effect as an advanced stage during final fate of injured cells inside the pancreas. The present findings were confirmed by [14] who noticed similar histological findings during development and progression of acute pancreatitis in rats.

Ultrastructurally, the pancreatic acinar cells in Group II had swollen mitochondria with dilated rough endoplasmic reticulum with decreased zymogen granules. These findings are like the results of some researchers [15], who found severe damage to the acinar cells and islets of Langerhans with subsequent acute pancreatitis in rats treated with another cytotoxic drug (puromycin). In addition, [16] who described the same changes occurred in the nucleus of the pancreas of rats treated with cyclosporine which were abnormalities in the nuclear membrane with disturbance in the chromatin matrix.

In this study, bleomycin sulfate could result in a picture of moderate to severe form of pancreatitis. These results could be partially explained by considering the mode of action of bleomycin sulfate. Some authors [2] mentioned that the bleomycin sulfate cleaves DNA by generating free radicals. In the presence of O₂ and a reducing agent, such as dithiothreitol, the metal-drug complex becomes activated and functions as a ferrous oxidase, transferring electrons to produce oxygen radicals. In addition, another work by [17] who found that bleomycin sulfate disturbs the normal structure of the pancreas specially islet cells with similar changes as in this study. Moreover, the functional parameters of pancreas after bleomycin treatment such as glucose transferring factors (GLUTF 1, 2), and Protein Kinase C, and A (PKC) and (PKA) were affected. These changes might be due to oxidative stress generated by bleomycin sulfate leading to
β-cells injury with subsequent development of type 2 diabetes, which predisposing to cell apoptosis and compromising the responsiveness of insulin-secreting cells.

In this work, bleomycin sulfate induced significant degree of damage on β-cells and α-cells with marked degranulation of their secretory granules with nuclear degeneration. These results were similar to the results of [15] who found that the cytotoxic drug (puromycin) in rats caused marked damage to the β-cells and α-cells with degranulation in their granules with subsequent development of diabetes. The β-cells degranulation and cytoplasmic vacuolization were likely to be the result of the inhibition of synthesis and secretion in β-cells under the effect of puromycin. Due to either reduced capacity to store insulin, or damage to the mechanism of insulin secretion.

In the present work, the morphometric result showed significant decrease in the mean area percentage immunostaining in group II which indicated that bleomycin producing significant injury to β-cells. This finding was in consistence with findings of [18] who reported that in the pancreas of diabetic rats the islet cells were shrunken with necrotic changes. Another study by [17] who found that bleomycin sulfate disturbed the normal structure of the pancreas predisposing to cell apoptosis specially islet cells.

In the current work Masson's trichrome stain showed increase in the collagen fibers deposition within the parenchyma of the pancreas. This result agreed with some authors [19-21] who observed that an increase in connective tissue of the lung including elastin fibers and other connective tissue fibers after bleomycin administration. This might be explained by [22] who found that bleomycin treatment significantly increased the mean number of myofibroblasts which were the principal cells responsible for deposition of collagen and extracellular matrix in lung fibrosis. The same finding observed by [23], who noticed increase in collagen fibers around the blood capillaries and between the islet cells after streptozotocin use.

Fortunately, Vitamin E supplementation to bleomycin sulfate treated rats resulted in alleviation of pancreatic injury. Most of the acini were improved with regeneration of islet of Langerhans which appeared in close associations with the ducts. These findings were in line with the work of [24] who found the same results in pancreas of rats treated with cyclosporine with Vitamin E. Who explained the regeneration of the islet from the duct after administration of Vitamin E by a process called neo-genesis or (budding formation) due to formation of primitive islets near the duct, which were actively proliferated and then herniated to the neighboring mesenchyme to form the new regenerated islet of Langerhans (secondary islet), these islets still retained their close relationship to the duct. Ultrastructurally, the acinar cell showed marked signs of recovery with improvement in zymogen granules in amount and in density. The mitochondria and nucleus restored to the nearly normal shape and size with regular nuclear membrane. Similar results were noticed by some authors [25,26], who found improvement of the liver by Vitamin E supplementation with acrylamide use. This effect may be related to the antioxidant properties of Vitamin E which decreased the free radical's formation and reduced glutathione levels in hepatocytes.

Furthermore, due to its anti-inflammatory effect some authors [27] stated that Vitamin E was the most potent antioxidant bio-membranes particularly with respect to lipid peroxidation. They added that its penetration to a precise site in the membrane might be an important feature of the protection against highly reactive radicals, also, Vitamin E had been shown to inhibit efficiently cellular drug accumulation inside the tissues. Also, some researchers [28] suggested that Vitamin E appeared to be the first line of defense against peroxidation of polyunsaturated fatty acids, which were present in cellular and subcellular membrane phospholipids. Vitamin E acting as antioxidant, breaking free radicals chain reactions which might be produced by the toxic agents on the tissues. This agrees with the work of [29] who reported that Vitamin E reduced DNA fragmentation and apoptotic cells formation. So, Vitamin E might act by maintaining prolonged cell cycle during which DNA repair in cases with thyroid dysfunction. Furthermore, [30] reported that Vitamin E supplementation improved the expression levels of several genes involved in free radicals scavenging resulting in decreasing DNA damage in cases of thyroiditis.

In this research administration of Vitamin E revealed significant increase in the mean area percentage immunostaining of the B-cells. Similar results were reported by [31] who found improvement in β-cells and α-cells nuclei with increasing in their secretory granules in rats that had taken Vitamin E concomitantly with Diazinon.

In Group III stained with MTC revealed nearly normal collagen fibers distribution in the blood vessel wall. The same results as the work of [32].
who found that the fibrosis in thyroid gland in rats treated with amiodarone decreased after administration of Vitamin E. He explained that Vitamin E inhibited lipid peroxidation and caused a decrease in collagen gene expression.

**Conclusion:**

The present data strongly confirm the undesirable effect of bleomycin sulfate on the pancreas of the adult male albino rats. However, pre-treatment with Vitamin E significantly protect the pancreas against bleomycin-induced adverse effects.

**References**


The Possible Protective Effect of Vitamin E on Pancreas


التأثير الوقائي لفيتامين ه على بنكرياس الفئران البالغة

الخلطية: البتايوسين سلفات هو مضاد حرفي للعلاج الكيميائي قد يكون له أثار ضارة على كل من أنسجة والقد الدماء في البنكرياس.

هذ الدراسة: صمت هذه الدراسة لتقدير تأثير البتايوسين سلفات على البنكرياس لدى ذكور الجرذان البيضاء، والحماية المكثة عن طريق إعطاء فيتامين ه.


المستنتاجات: أظهر العلاج البتايوسين سلفات تغيرات نسيجية مرضية متغيرة. تظهر البنية الطبيعية لنسج البنكرياس مع مناطق النزف والفراغ والل-notes في الخلايا الغنية البنكرياسية أو جزر البنكراز مع فقدان التباين بين خلايا ببتا وخلايا آلفا. تضاع فاقت النسب المرضية الخلايا الغنية البنكرياسية أكثر إتلافياً مع النوى الفاقية المستدورة وفصيولات الدم الحمضى القمي. استعادت جزيرة البنكراز شكلها الطبيعي وحجمها بخط خارجي مظلم.

الخلاصة: يمكن لفيتامين ه أن يحمي البنكرياس من التغيير الناجم عن كريبيتي البتايوسين في ذكور الجرذان البيضاء.