

EFFECTS OF DIETHYLDITHIOCARBAMATE ON DIABETOGENIC ACTION OF ALLOXAN IN
GUINEA-PIGS : AN ULTRASTRUCTURAL STUDY*

KOBAYLARDA ALLOKSANIN DİYABETOJENİK TESİRİNE DİETİLDİTİYOKARBAMATIN
ETKİSİ : BİR ULTRAMİKROSKOBİK ÇALIŞMA

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In attempts to induce diabetes in guinea-pigs by alloxan, a diabetes-like state appears after injection, but this situation is followed by islet β -cell regeneration and insulin insufficiency disappears within two weeks. In order to test whether diethyldithiocarbamate (DDC), a superoxide dismutase (SOD) inhibitor, enhances diabetogenic action of alloxan in guinea-pigs, we divided guinea-pigs into three groups, as follows: (i) saline plus saline treated controls, (ii) saline plus alloxan treated guinea-pigs (ALL) and (iii) DDC plus alloxan-treated guinea-pigs (DDC+ALL). After an overnight fast, DDC (750 mg/kg) or saline were injected intraperitoneally to the guinea-pigs. After 2.5 hours, alloxan (200 mg/kg) or saline were injected to the guinea-pigs by intracardiac route. 7 and 14 days after injections, the guinea-pigs were weighed, fasting serum glucose and oral glucose tolerance testing (OGTT) were performed. After sacrifice, light and electron microscopic investigations of pancreas were done. Alloxan stopped weight gain and increased glucose levels slightly 7 and 14 days after the injection. Additional DDC administration decreased the weight gain and increased the glucose levels further but not significantly. 7 days after the injection, while alloxan increased the OGTT index slightly, DDC addition significantly increased the index. 14 days after the injections, we observed no significant effect of the treatments on the OGTT index. Our light and electron microscopic findings were in parallel with our biochemical findings. The islets of Langerhans were smaller in ALL and DDC+ALL groups than those in the control group. α and δ cells at the islets remained unaltered, while β -cells decreased in number. At the 14th day, the islets of the ALL and DDC+ALL treated guinea-pigs appeared almost normal. These results suggest that, guinea-pigs are not susceptible to diabetogenic action of alloxan, even if cellular SOD is inhibited. So, the mechanism of this resistance may depend on other enzymatic pathways which differs between guinea-pigs and other alloxan-susceptible species.

Kobaylarda, alloxan ile diyabet oluşturma girişimlerinde enjeksiyondan sonra diyabet oluşmakta, fakat bu durumu adacık β -hücre rejenerasyonu takip etmekte ve insülin yetmezliği iki hafta içinde ortadan kalkmaktadır. Bu çalışmada, bir süperoksid dismutaz inhibitörü olan diethyldithiocarbamate (DDC)'in kobaylarda alloxanın diyabetojenik etkisini artırıp artırmadığını test etmek amacıyla kobaylar, (i) serum fizyolojik (SF)+SF kontroller, (ii) SF+alloksan uygulanan kobaylar (ALL) ve (iii) DDC+alloksan uygulanan kobaylar (DDC+ALL) olmak üzere üç gruba ayrılarak kobaylardaki resistansın mekanizması araştırıldı. Gece boyu açlık periyodunu takiben, hayvanlara DDC (750 mg/kg) veya SF intraperitoneal olarak uygulandı. 2.5 saat sonra ise, alloxan (200 mg/kg) veya SF intrakardiyak olarak verildi. Enjeksiyonlardan 7 ve 14 gün sonra, kobaylar tartıldı, açlık kan glukozları ölçüldü ve oral glukoz tolerans testleri (OGTT) yapıldı. Hayvanlar öldürüldükten sonra, pankreasları ışık ve elektron mikroskopik olarak incelendi. Alloxanın, 7. ve 14. günlerde ağırlık artışını engellediği ve glisemi değerlerini hafifçe artırdığı gözlemlendi. İlave olarak DDC uygulamasının ise anlamlı olmamak üzere, ağırlık artışını daha fazla engellediği ve glisemi değerlerini biraz daha artırdığı ve DDC eklenmesi ile OGTT'deki yükselmenin anlamlı derecede olduğu gözlemlendi. 14. günde ise alloxan ve/veya DDC uygulanmasının herhangi bir anlamlı etkisi gözlenmedi. Işık ve elektron mikroskopik çalışmaların bulguları da bu bulgularımızla uyumlu idi. ALL ve ALL+DDC gruplarında Langerhans adacık hücreleri kontrollere göre daha küçüktü. α ve δ hücreleri değişmezken, β -hücrelerinin sayısında azalma görüldü. 14. Günde ise ALL ve DDC+ALL gruplarının adacık hücreleri normal görünümde idi. Bu sonuçlara göre, kobaylar alloxanın diyabetojenik etkisine, hücrel süperoksid dismutaz inhibe edilse dahi, hassasiyet göstermemektedirler. O halde, kobaylardaki bu resistansın mekanizması kobaylar ve alloxana duyarlı türler arasında farklılık gösteren diğer yollara bağlı olabilir.

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Keywords : *Diabetes; Alloxan; Diethyldithiocarbamate; Superoxide dismutase*

Anahtar Kelimeler : *Kobay; Diyabet; Alloksan; Diethyldithiocarbamate; Superoksid dismutaz*

Introduction

A single injection of alloxan, or alloxan like derivatives of uric acid, destroys the insulin-producing islet β -cells and causes diabetes mellitus in most animal species like dogs, cats, sheep, rabbits, rats, monkeys, fish, turtles, birds, and some, but not all, hamster species (1). Interestingly, guinea-pigs appear to be immune to alloxan-induced diabetes and the reason for this lack of susceptibility is unknown (1). In attempts to induce diabetes in guinea-pigs by alloxan, a diabetes-like state appears after injection, but this situation is followed by islet β -cells regeneration and insulin insufficiency disappears within two weeks (2).

Cu-Zn Superoxide dismutase (SOD), an enzyme removing superoxide anion radicals, has been reported to protect the islet cell function from damage of alloxan in mice and rats (3-6). Diethyldithiocarbamate (DDC), a copper chelating agent and SOD inhibitor (7), has been reported to enhance diabetogenic action of alloxan in rats (5). In view of these findings, it has been postulated that decreased SOD activity in β -cells increases the susceptibility to diabetogenic action of alloxan (3-6). It has been reported that alloxan limitedly inhibits Cu, Zn SOD of man, dog, rat and cow (8). Hydrogen peroxide produced during the metabolism of alloxan was described as being responsible for this enzyme inactivation (9). However, it has recently been reported that alloxan produces hydrogen peroxide radicals in β -cells, but it does not effect SOD levels in rats (5).

Our knowledge is little about the role of SOD in this resistance of guinea-pigs to alloxan diabetes. The objective of this study, therefore, was to test whether SOD inhibition enhances diabetogenic action of alloxan in guinea-pigs.

Materials and Methods

Guinea-pigs (n=28), weighing 483-589 g, were divided into three groups, as follows: (i) saline plus saline treated controls (n=6), (ii) saline plus alloxan treated gui-

nea-pigs (ALL, n=12) and (iii) DDC plus alloxan-treated (DDC+ALL, n=12) guinea-pigs. Food was routinely withheld 15 hours prior to DDC or saline administration. DDC (750 mg/kg) or proper volumes of saline were injected intraperitoneally to the fasted guinea-pigs. After 2.5 hours, alloxan (200 mg/kg) or proper volumes of saline were injected by intracardiac route. Food was returned 1 hour after the injection. Each ALL and DDC+ALL groups were further divided into two (7 and 14 days, 7D and 14D) groups (n=6 each) as to compare the effects of the injections at the first and the second week. 7 and 14 days after injections, the guinea-pigs were weighed, food was withheld for 16 hours and blood samples were obtained just prior to the oral administration of glucose (2 g/kg body weight) for oral glucose tolerance test (OGTT). Additional blood samples were obtained by clipping the toenails one hour and four hours after the glucose load. Glucosticks (Ames, Bayer) were used to determine the amount of glucose in each of these samples. The one- and four- hour OGTT values were averaged in the following manner: The difference between the one-hour and fasting blood glucose (FBG) levels were added to three times the difference between the four-hour OGTT and the FBG value, and the sum was divided by four [(1 hour - FBG) + 3(4 hour - FBG)]

4. Any number over 100 was regarded as distinctly abnormal. Body weight index was calculated by dividing the values at the end of the follow up by the initial body weights. One way analysis of variance (ANOVA) and post hoc Duncan's multiple range test was used for statistical comparisons. A p level < 0.05 was considered significant.

At the time of killing, three pieces of the splenic lobe of each pancreas were removed and fixed in phosphated buffer 2% glutaraldehyde (Saronsen) solution. Then, routine procedure for electron microscopy was followed up. Semi-thin sections were stained with methylene Blue-Azur II for light microscopic examinations. Ultra-thin sections were stained with uranyl acetate and lead citrate for electron microscopic investigations which were performed and photographed by using a Zeiss EM9S. All chemicals and solvents used were of analytical grade.

Results

Effects of alloxan and alloxan + DDC treatments Alloxan stopped weight gain and increased glucose levels in 7D and 14D groups slightly (p > 0.05 vs. control, respectively), but guinea-pigs gained weight and glucose levels were slightly lower in the control groups.

Table. The effects of alloxan and DDC+alloxan treatments

Groups	DDC+Alloxan		Alloxan		Control	
	7th Day	14th Day	7th Day	14th Day	7th Day	14th Day
Body Weight Index (% of initial)	96.83+3.91	95.06+12.02	100.16+2.32	100.71+1.76	104.69+2.74	109.65+5.37
Fasting Blood Glucose (mg/dl)	138.66+7.12	130.00+17.21	105.11+11.11	109.25+7.10	96.75+8.97	98.67+9.68
OGTT Index	88.83+7.20*	51.00+2.98	70.75+12.60	46.00+8.56	57.93+11.07	58.67+ 12.76

DDC= Diethyldithiocarbamate

* p<0.05 vs. control at 7th day.

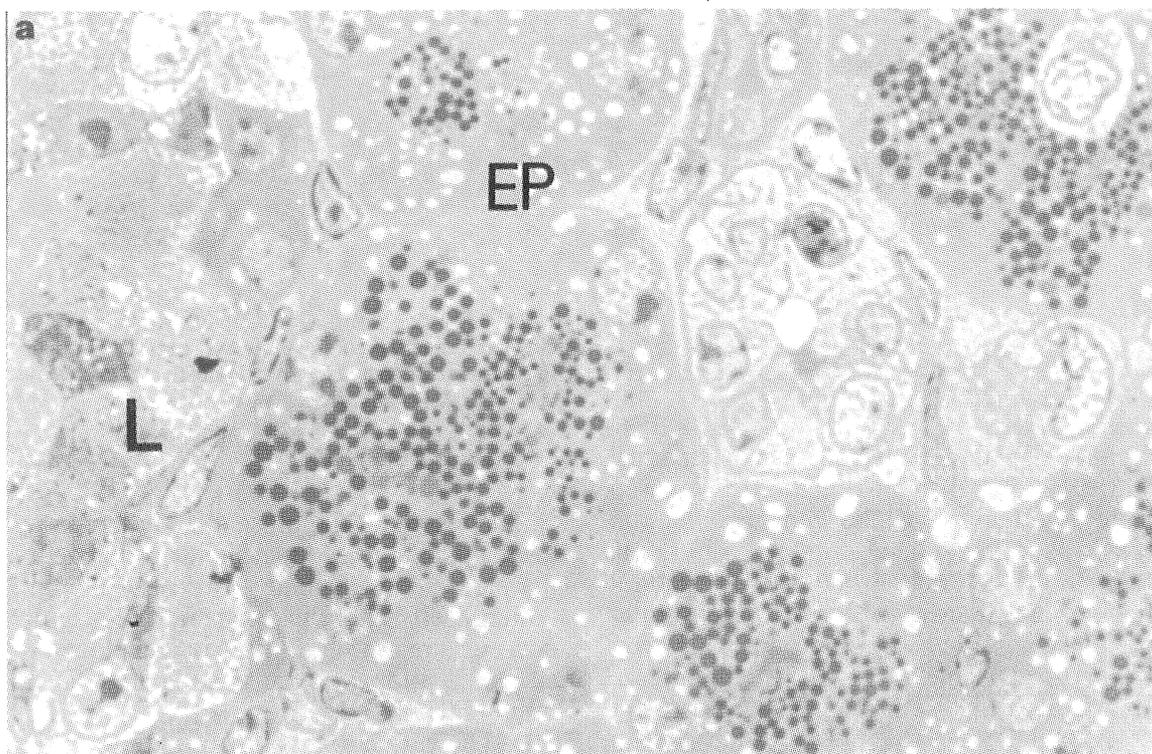


Fig.1a. Plastic sections (light micrograph) of the pancreas from ALL groups at the 7th day. In DDC+ALL group, degenerative acinar cells (arrows) that were characterized with picnotic nucleus, decreased zymogen granules and widening structures in cytoplasm are seen. EP: Exocrin pancreas, L: Islet of Langerhans (Methylene blue-azur II; a,b:X120)

Additional DDC administration decreased the weight gain and increased the glucose levels further but not significantly (Table).

In 7D group, alloxan increased the OGTT index slightly. However, DDC addition significantly increased the index (p<0.05 vs. control). In 14D groups, we observed no significant effect of the treatments on the index (p>0.05 vs. control, respectively) (Table).

Light and electron microscopic findings after treatments

Our light and electron microscopic findings were in parallel with our biochemical findings in 7D groups. The islets of Langerhans were smaller in ALL and DDC+ALL groups than those in the control group. α - δ cells at the islets remained unaltered, while β -cells

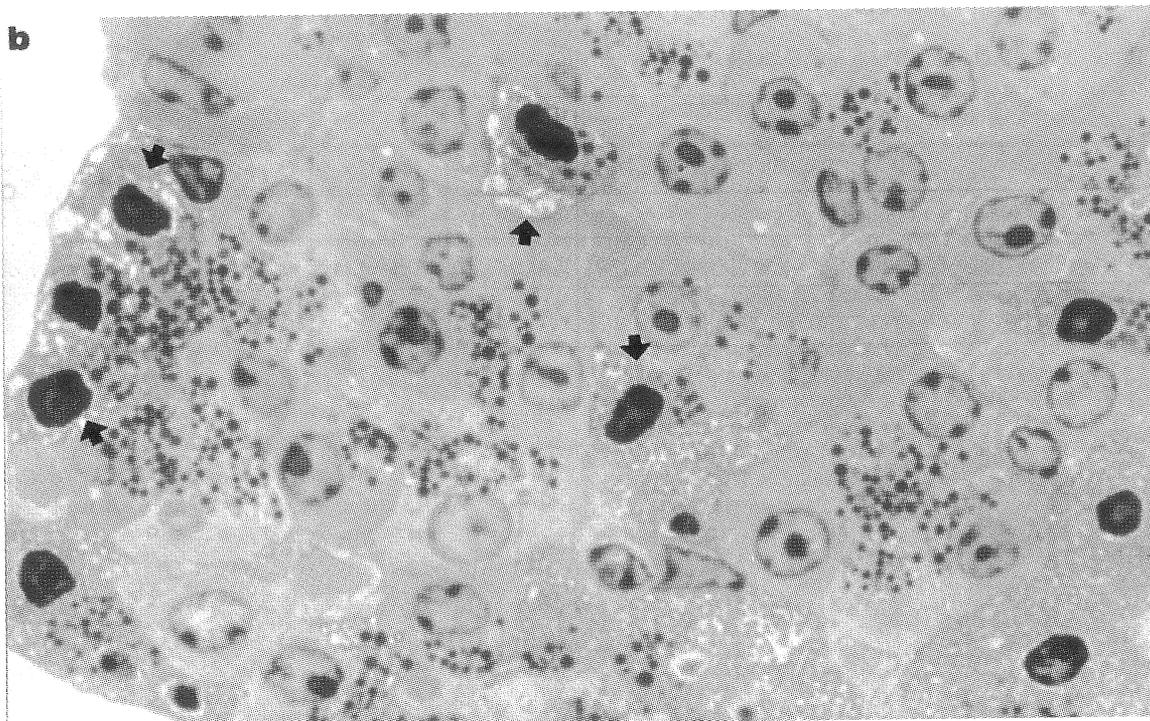


Fig. 1b. Plastic sections (light micrograph) of the pancreas from DDC+ALL groups at the 7th day. In DDC+ALL group, degenerative acinar cells (arrows) that were characterized with picnotic nucleus, decreased zymogen granules and widening structures in cytoplasm are seen. EP: Exocrine pancreas, L: Islet of Langerhans (Methylene blue-azur II; a,b:X120)

decreased in number, having dilated individual profiles of endoplasmic reticulum, decreased secretory granules (which were not electron dense) and vacuolated mitochondria (Figs. 1,2). In DDC+ALL groups, the structural changes in endocrine pancreas were similar to those observed in ALL groups. However, at the 7th day of DDC+ALL treatment, we investigated that additional DDC treatment increased the toxic effect of alloxan treatment on pancreatic β -cells. At the 14th day, the islets of the ALL and ALL+DDC treated guinea-pigs appeared almost normal (Fig. 3).

In addition, we observed that the exocrine pancreas has also been negatively affected by DDC+ALL treatment. At the 7th day, we noticed lipid droplets in the cytoplasm of pancreatic acinar cells which had picnotic nuclei, a marked decrease in the number of the zymogen granules, otophagic vacuoles which had myelin figures, manifest dilatation in endoplasmic reticulum and cristae destruction in mitochondria (not shown). These pathological changes were further increased at the 14th day (Fig.4).

Discussion

The results of the present study indicates that DDC-treated rats show no change in diabetogenic susceptibility to a dose of alloxan, which itself is not diabetogenic. Thus, guinea-pigs are not susceptible to diabetogenic action of alloxan, even if cellular SOD is inhibited.

The guinea-pig is one of the few species that appears to be resistant to the diabetogenic action of alloxan (1,2). Morphological studies have suggested that the pancreatic β -cells of the guinea-pig are destroyed by alloxan, however, diabetes may not occur because the β -cells population subsequently regenerates from either intraislet and extraislet sources. In a previous study, it has been reported that alloxan produces a diabetes-like condition early after injection but all signs disappear within two weeks, by which time serum insulin levels and the volume fraction of β -cells in the pancreas have returned

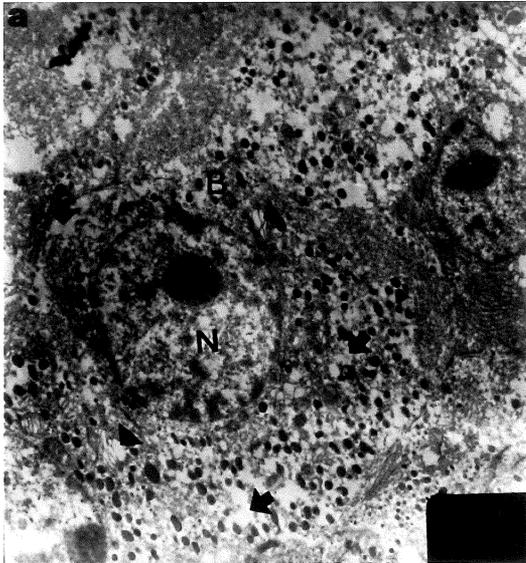


Fig.2a. Electron micrographs of the islets of Langerhans from the ALL groups at the 7th day. The presence of the dilatation of the endoplasmic reticulum cisternae (arrowheads) and the wide electron lucent spaces (arrows) in the cytoplasm of the B cells (B) are shown. N:Nucleus. (Original magnification: a: X5000) Uranyl acetate and Reynold's lead stain

to normal. However, streptozotocin treatment of guinea-pigs causes a diabetes like condition which is not reversed later. It has been postulated that regeneration of islet β -cells following destruction by alloxan may be the primary cause of recovery of alloxan-injected guinea pigs from the effects of the drug (10).

SOD is an enzyme which removes superoxide anion radicals. It has been reported that SOD is prophylactic against alloxan diabetes in mice and rats (3-6). Considering alongside the recent finding (5) that DDC enhances diabetogenic action of alloxan in rats, it has been postulated that SOD activity is important in the viability of β -cells. It has been reported that alloxan produces hydrogen peroxide

radicals (5-11) in β -cells, but it does not effect SOD levels (5). However, streptozotocin (STZ) not only produces hydrogen peroxide radicals

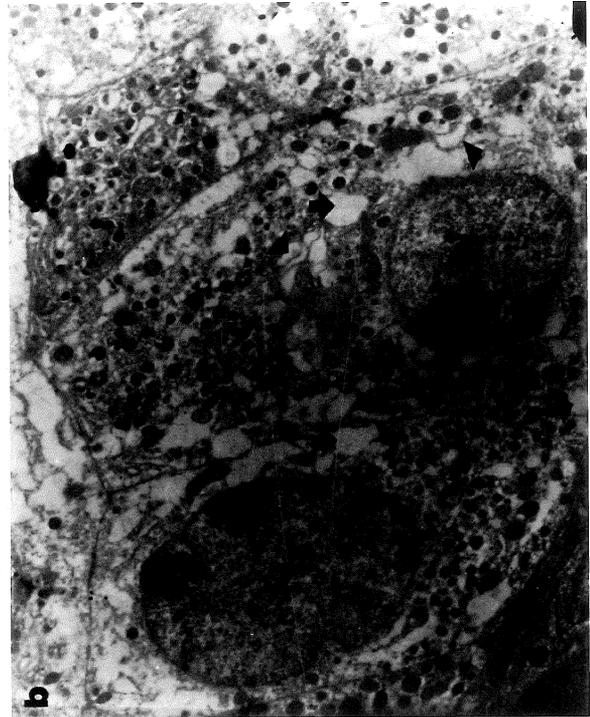


Fig.2b. Electron micrographs of the islets of Langerhans from the DDC+ ALL groups at the 7th day. The presence of the dilatation of the endoplasmic reticulum cisternae (arrowheads) and the wide electron lucent spaces (arrows) in the cytoplasm of the B cells (B) are shown. N:Nucleus. (Original magnification: a: X 5000) Uranyl acetate and Reynold's lead stain

in β -cells (11) but also it decreases cellular SOD levels. STZ is effective in acute chemical induction of diabetes in the male guinea-pig (12). In STZ-treated guinea-pigs, a persistent insulin deficiency occurs which is not reversed by islet cell regeneration later (10). So, lack of an additional direct effect of alloxan on SOD may be the reason of the resistance of guinea-pigs to alloxan diabetes. However, we could not support this hypothesis in our study. In the present study, neither alloxan nor its combination with DDC produces any diabetic state in guinea-pigs, as confirmed by both biochemical and morphological findings. The increase in OGTT index in DDC+ALL group was not enough to be

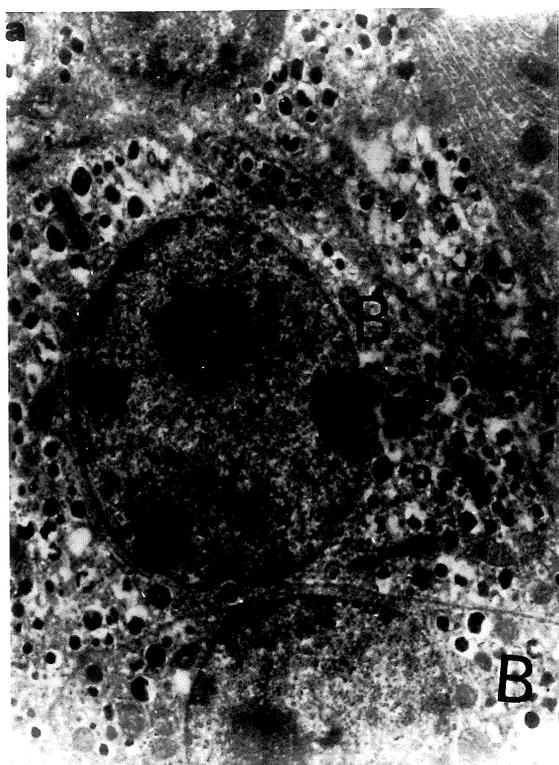


Fig.3a. At the 14th day, transmission electron micrographs of islets of Langerhans of the guinea pigs from the ALL groups, showing cell types labeled A and B, with their characteristic granules. (Original magnification:a:X 5000) Uranyl acetate and Reynold's lead stain.



Fig.3b. At the 14th day, transmission electron micrographs of islets of Langerhans of the guinea pigs from the DDC+ALL groups, showing cell types labeled A and B, with their characteristic granules. (Original magnification:b:X6000) Uranyl acetate and Reynold's lead stain

accepted as a diabetic state.

In this study, destruction of β -cells were more prominent in the islets of Langerhans 7 days after DDC+ALL treatment than that in the alloxan group. Other interesting finding in this study were a decrease in the number of zymogen granules and degenerations such as picnotic nucleus, dilatation of the endoplasmic reticulum cistarnea, increased lipid droplets, destruction of mitochondria in pancreatic aciner cells. Since the alloxan groups did not show such pathological changes, these findings in DDC+alloxan group may be due to the effect of DDC.

Numerous isoenzymes of the Cu-Zn-SOD have been described in mammals with varying tissue distribution. The possibility that the isoenzyme present in islets of guinea-pigs may not be susceptible to alloxan inhibition

can be excluded; because DDC inhibits SOD but it has no influence of the susceptibility of the animals to alloxan-diabetes.

To our biochemical and morphological results, additional DDC treatment slightly increased the susceptibility of the guinea-pigs to diabetogenic effect of alloxan at the 7th day. However, this effect of DDC was decreased at the 14th day.

Taken as a whole, guinea-pigs are not susceptible to diabetogenic action of alloxan, even if cellular SOD is inhibited. So, the mechanism of this resistance may depend on other enzymatic pathways which differs between guinea-pigs and other alloxan-susceptible species. Further investigations are needed to make the machanism of this resistance clear.

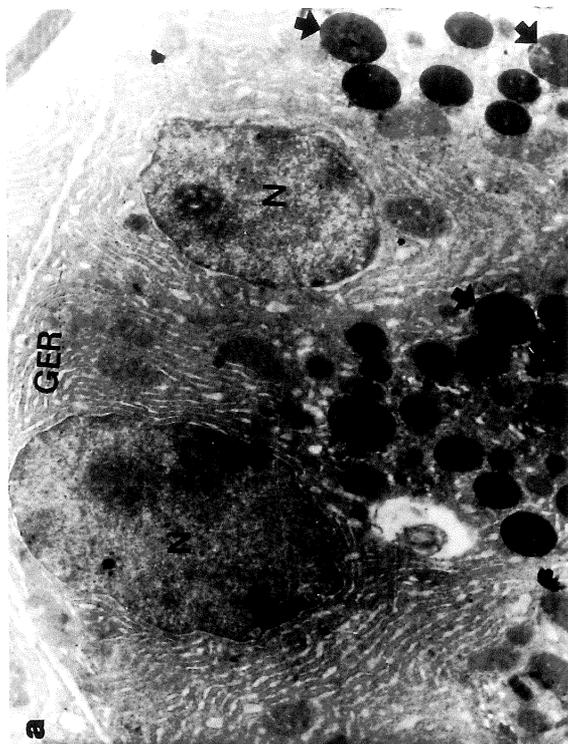


Fig.4a. Electron micrographs of guinea pigs exocrine pancreas from the ALL groups at the 14th day. In the DDC+ALL group, as parallel with our light microscopic findings, degenerative pancreatic acinar cells are seen. N: Nucleus; arrowheads: lipid droplets; GER: granules endoplasmic reticulum; thin arrows: vacuoli; thick arrows: zimogen granules. (Original magnification:a:X3000; b:X5000) Uranyl acetate and Reynold's lead stain

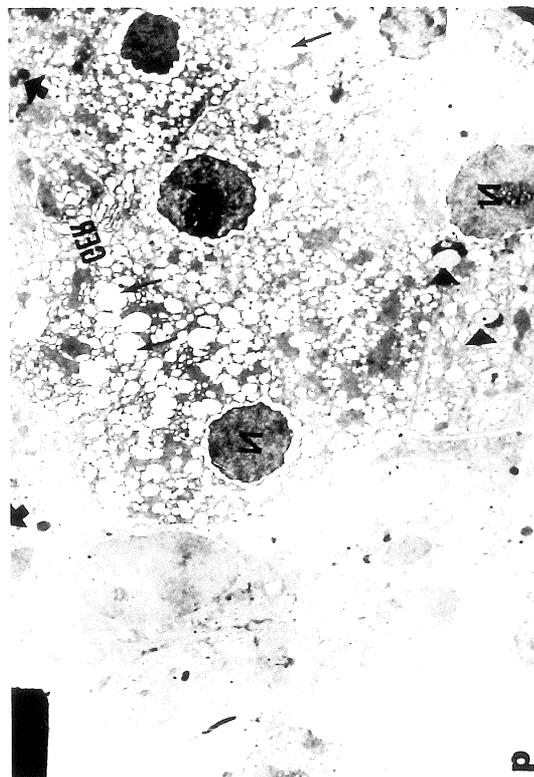


Fig.4b. Electron micrographs of guinea pigs exocrine pancreas from the ALL groups at the 14th day. In the DDC+ALL group, as parallel with our light microscopic findings, degenerative pancreatic acinar cells are seen. N: Nucleus; arrowheads: lipid droplets; GER: granules endoplasmic reticulum; thin arrows: vacuoli; thick arrows: zimogen granules.. (Original magnification:a:X3000; b:X5000) Uranyl acetate and Reynold's lead stain

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