EFFECT OF D-PENICILLAMINE INDUCED COPPER DEPLETION ON RAT LUNG ELASTIN CROSS-LINKING DURING THE PERINATAL PERIOD

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D-PENİSİLAMİN İLE İNDÜKLENMİŞ BAKIR DEPLESYONUNUN PERİNATAL DÖNEMDE SIÇAN AKCİĞERİ ELASTİN ÇAPRAZ BAĞLARI ÜZERİNE ETKİSİ

Özet: *Bu çalışmada, çeşitli patolojilerin tedavisinde kullanılan bir farmakolojik ajan olan D-Penisilamin'in (DPA) perinatal süreçte kullanılmasının, bakır deplesyonu oluşturması nedeni ile yenidoğan akciğer elastin oluşumunu ve gelişimini etkileyip etkilemediği araştırıldı. Çalışmaya 40 dişi (n=20 kontrol grubu, n=20 deney grubu) 6 erkek rat'ın çiftleştirilmesi ile elde edilen 20 yenidoğan rat alındı. Deney grubunu oluşturan maternal ratlara gebelik süreçlerinin 14. gününden itibaren, tüm emzirme sürecini de kapsayan postnatal 21. güne kadar 400 mg/kg/gün DPA 3 ml serum fizyolojik içinde çözündürülerek intraperitoneal yoldan verildi. Kontrol grubundaki maternal ratlara (n=20) deney süreci boyunca (prenatal 14. gün ile postnatal 21.gün arası) intraperitoneal serum fizyolojik enjeksiyonu yapıldı. Deney sürecinin sonunda 20 yenidoğan, 10 adet maternal kontrol grubundan, 10 adet maternal deney grubundan olmak üzere rastgele seçildi. Kontrol ve deney grubu arasındaki gelişim farklılığı yenidoğanların vücut ve akciğer ağırlıkları tartılarak belirlendi. Bakır deplesyonunun düzeyinin belirlenmesi için maternal ve yenidoğan gruplarında serum bakır düzeyleri ve seruloplazmin aktiviteleri saptandı. Akciğer elastin düzeyi ve elastine spesifik çapraz bağ amino asidleri olan desmosine (DES) ve isodesmosine (IDES) düzeyleri yenidoğan kontrol ve deney gruplarında saptandı. Serum bakır düzeyi atomik absorpsiyon spektrofotometresi, serum seruloplazmin aktiviteleri spektrofotometrik yöntem kullanılarak belirlendi. Akciğer dokusu DES, IDES ve elastin düzeylerinin saptanması için yüksek performanslı sıvı kromatografisi kullanıldı. Elde edilen sonuçlar, deney grubuna perinatal ve postnatal süreçte DPA verilmesinin bakır deplesyonu oluşturduğunu, deri elastisitesinde, vücut ve akciğer ağırlıklarında azalmaya yol açtığını gösterdi. Maternal ve yenidoğan deney grubunda serum bakır düzeyleri ve seruloplazmin aktivitesi sırasıyla 24.33±3.05mg/dl, 16.31±2.96 U/L and 11.70±1.75mg/dl, 3.50±0.76U/L saptandı. Maternal ve yenidoğan kontrol grubunun serum bakır düzeyleri ve seruloplazmin aktivitesi sırası ile 64.38±7.38mg/dl, 54.05±4.81U/L, 55.44±4.56mg/dl, 22.96±2.26U/L olarak belirlendi. Yenidoğan kontrol grubunda saptanan DES, IDES ve elastin düzeyleri 24.78±12.81mg/g, 7.16±1.50mg/g, 170±29.92mg/g yaş doku, deney grubunda 10.90±3.83mg/g, 4.90±1.58mg/g, 90.57±24.62mg/g yaş doku olarak saptandı. İstatistiksel değerlendirmede gruplardaki örnek sayısına bağlı olarak Student-t test ve Mann -Whitney U testleri kullanıldı. Deney ve kontrol grupları arasında bakır düzeyleri ve seruloplazmin aktivitesi yönünden istatistiksel olarak anlamlı farklılık saptandı ($p<0.001$). Yenidoğan deney grubunun akciğer dokusu DES, IDES ve elastin düzeylerinde kontrol grubuna göre anlamlı bir düşme olduğu gözlendi ($p=0.0004$, $p=0.0020$, $p=0.05$). Sonuç olarak oluşturduğumuz deney modeli, prenatal ve postnatal süreçte kullanılan DPA'nın yenidoğan akciğer dokusunda elastin çapraz bağ oluşumunu ve yenidoğan gelişimini olumsuz etkilediğini göstermektedir.*

Anahtar Kelimeler: *Desmosine, D-Penisilamin, İsodesmosine, Perinatal dönem*

Summary: *This study was designed to clarify whether D-Penicillamine (DPA), a drug used for treatment of various pathological events, induced copper depletion effects on lung elastin formation and maturation of the newborn in the perinatal period. The investigation was conducted on 20 newborn rats breaded from 40 female and 6 male rats. DPA (400*

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mg/kg/day) was administered to 20 maternal rats intraperitoneally (i.p) beginning on the 14th day of gestation until ending on the postnatal 21st day during the all-suckling period. In the same period physiologic saline was administered to 20 maternal rats to obtain control group. At the end of the experimental period, 20 newborn rats were selected randomly from each breeder, 10 from control and 10 from experimental group. The body and lung weights of newborns were assessed. Serum copper (Cu) levels and ceruloplasmin (Cp) activities of the maternal and newborn groups and lung tissue elastin, desmosine (DES) and isodesmosine (IDES) levels in the newborn groups were measured with comparing with the control groups. Atomic absorption spectrophotometer and spectrophotometric methods were used for Cu and Cp measurements respectively. High Performance Liquid Chromatography (HPLC) was used for the determination of DES and IDES, the specific cross-linking amino acids in elastin, and elastin levels. The results showed that DPA induced copper depletion caused loss of skin elasticity and reduction in body and lung weight in experimental newborns group. The serum Cu levels and Cp activity in maternal and newborn experimental groups were found 24.33±3.05mg/dl, 16.31±2.96 U/L and 11.70±1.75mg/dl, 3.50±0.76U/L respectively. In the maternal and newborn of control groups Cu and Cp values were 64.38±7.38mg/dl, 54.05±4.81U/L, 55.44±4.56mg/dl, 22.96±2.26U/L respectively. The lung DES, IDES and elastin values of control were 24.78±12.81mg/g, 7.16±1.50mg/g, 170±29.92mg/g wet-tissue and in experimental groups were 10.90±3.83mg/g, 4.90±1.58mg/g, 90.57±24.62mg/g wet-tissue respectively. The statistical evaluations of data were made using Student-t and Mann-Whitney U tests according to the number of groups. We found significant difference for Cu levels and Cp activities between control and experimental groups ($p<0.001$). DES, IDES and elastin levels showed significant decreases in experimental newborn group compared with the control group ($p=0.0004$, $p=0.0020$, $p=0.05$). In conclusion; our experimental model includes both gestational and postnatal periods and this model may be useful for working with the effect of DPA induced copper depletion on the maturation of the newborn connective tissue. Another conclusion drawn from this study is that Cu depletion in middling quality due to DPA administration induces change in cross-linking in lung elastin during the perinatal period.

Key Words: *Desmosine, D-Penicillamine, Elastin, Isodesmosine, Perinatal period.*

INTRODUCTION

D-Penicillamine (dimethylcysteine) is a degradation product of penicillin and is prepared by its hydrolysis. Only the D isomer of penicillamine is recommended for clinical application and is used mainly in the therapy of Wilson's disease (1-3), cystinuria (4) and rheumatoid arthritis (5). DPA is also an effective chelator of copper (Cu), mercury, zinc and lead, promoting the excretion of these metals in urine (6). The wide use of DPA in human therapy and the reports in the literature associated with pathological events have stimulated a series of experimental studies on the effect of this drug on various organs and systems (7 - 12). Elastin is a rubber-like insoluble protein presents in mammalian connective tissue (13,14) mostly, ligaments, blood vessels and lung. The extreme insolubility of elastin is due to the presence of covalent cross-links between groups on the side chains of lysine residues, which lie in proximity to adjacent polypeptide chains (15). DPA-treated weaning rats ave been demonstrated to contain less mature elastin than normal rats (16-18).The effect of DPA on elastin cross-linking is associated with its being a chelating drug and Cu being an important cofactor for lysyl oxidase (19). Desmosine (DES) and Isodesmosine (IDES) are specific cross-linking amino acids and isomers formed by the condensation of three-aminoadipic acid d-semialdehyde (allysine) residues with one lysine residue in elastin (20) and a number of investigations related to the cross-linking in various pathologies have been published in literature (21 - 23). Elastic and collagen fibers confer recoil and tensile strength to the pulmonary vasculature, airways, alveolar walls, and pleura. These durable extracellular matrix components are primarily synthesised during lung development and growth, and are expressed at very low levels in adult rat lung (24). DPA is the most commonly long-term used drug in some metabolic diseases such as Wilson, scleroderma (25), and rheumatoid arthritis, its usage in pregnancy is a controversial issue because of an association with a generalised connective tis-

sue defect similar to the Ehler-Danlos syndrome in neonates (26). In the extensive literature database search accomplished, only one citation was found related to its effect on elastin metabolism in the perinatal period in rat lung (16). This study was carried out to investigate the effects of DPA administered in the perinatal period and observe the formation and maturation of the lung elastin of the newborn. The design of the experiment, the model and the technique used in this investigation and the evaluation of the results as well as the results themselves are significantly different from those of Dubick et al. (18), as will be further discussed.

MATERIAL AND METHODS

Experimental design: This experiment was conducted on 40 newborn rats (Rattus norvegicus "Albinos L", F5 generation with homogeneity of 87.5 percent) of which 20 formed the experimental group, and 20 the control group. The breeding of these newborn rats was effectuated as follows: 40 female and 6 males were used as breeders. The beginning of the oestrus was assessed by the microscopically investigation of the vaginal fluid, and those females with manifest oestrus signs were left with a male in individual cages for a 24-hour period (28), the end of which was accepted as the 1st day of gestation. We planned to administer DPA on maternal rats intraperitonealy (i.p) at the late intra-uterine period and, to continue it during the early post-natal periods, when the liver Cu level in the newborn is reported to be nearer to the adult level (27). The experimental group of maternal rats was given a daily DPA dose of 400 mg/kg (i.p), dissolved in 3 ml of physiological saline, beginning on the 14th day of gestation and ending on the postnatal 21st. DPA dosage was determined in a previous study (29), in which it was observed that it caused 58 percent copper depletion in the maternal serum compared with the control group. The control group was given only 3 ml of physiological saline (i.p) daily throughout the experimental period.

The experiment rooms were fully air-conditioned with a temperature of 21±1°C and with a relative humidity of 60±5 percent, in 12 hour day/night cycles. The Animal Ethics Committee of Experimental Animal Research Center of Dokuz Eylül University, School of Medicine, approved the study protocols for the animal experiments.

Sampling: To note the physical changes visual observations were performed at the 7th day of postpartum related with skin elasticity. The lung and body weights were measured for both the control and experimental groups' offspring at the end of the experimental period. Measurements of the lung and body weights were performed using a balance (Sartorious-500ã), the results of which were used to assess the growth process of the newborn. Venous blood (from v.cava caudalis) samples were obtained from the newborn rats under Ketamin©-HCl anesthesia (80 mg/kg, i.p) and total lung tissues were obtained following cervical dislocation. All blood samples were centrifuged and the sera separated and stored in acid-washed polypropylene tubes at -70°C and the lung tissues were washed with physiological saline, blotted and stored at -70°C until analysis, not later than four months.

Chemical Analyses: Serum Cu levels were measured using a flame atomic absorption spectrophotometer (Shimadzu, AA-680ã) with a background corrector, according to Delves (30). Klayman and Neibur's spectrophotometric method was used to determine the Cp activity (31).

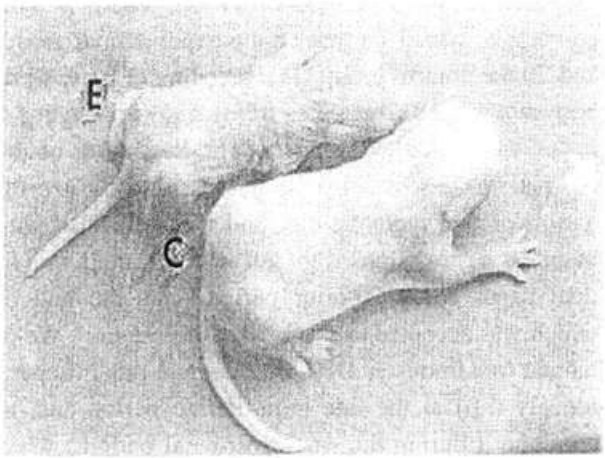
Analysis of DES and IDES in newborn lung tissues: Approximately 100 mg of wet lung tissue was employed for each analysis according to Reiser's method (32). The specimens were minced with scissors until the particle size was about 5mm. The tissues were homogenised in distilled water (1/5 volume ratio) and centrifuged at 1000 g for 5 min. The supernatant was decanted and 5ml of 0.1M NaOH were added to the pellet for resuspension. The mixture was boiled for 1 hour and pelleted by centrifugation; then washed twice with distilled water. The pellets were hydrolysed in 6N HCl for 24 hour at 110°C. The hy-

drolysates were pre-treated using the cellulose "micro-column" method, as reported by Skinner (33). The columns (1x5cm) consisted of 4-5ml of slurry of microgranular cellulose, (CF1) (10g) which was suspended in the mobile phase (n-butanol/acetic acid/water, 4:1:1, 200 ml). Hydrolysates were prepared by mixing with 2 ml of n-butanol, 0.5 ml of acetic acid and 0.5 ml of the additional cellulose slurry. Each of the prepared sample mixture was applied on a pre-conditioned column and passed through the column at a rate of 1-2 ml/min. Each column was washed twice with 8 ml of n-butanol:acetic acid: water (4:1:1) and completely drained. All the columns were eluted with 5 ml of distilled water into polypropylene tubes and the elutes were lyophilised under full vacuum and the residues were dissolved in 50ml of distilled water. Aliquots of the hydrolysates were analysed for their DES and IDES content by HPLC using a rapid isocratic elution system. A C18-reversed phase column (Shimadzuã, 0.4x10 cm) was used. The buffer contained 0.2 % SDS, 23% n-propanol , 5mM NaPO4, pH:3.3; the flow rate was 0.8 ml/min. DES and IDES were detected by their UV absorption at 275 nm (Shimadzuã BPD-6A) and compared with standards (Elastin Products Co. No: D866, D975).

Analysis of elastin in newborn lung tissues: Newborn lung elastin was prepared by Lansing's (34) procedure which involved heating of 200 mg minced or milled lung tissues in 0.1 N NaOH at 100°C for 45 minutes. After a wash with cold NaOH followed by distilled water, the elastin residue was dehydrated in a chloroform:methanol (2:1) mixture, air dried, and then brought to constant weight over P2O5 and weighed. Newborn rat lung elastin was rehydrated in 0.1M Tris, pH 7.5 and digested with thermolysine at 55°C for 4 hours using an estimated enzyme-substrate ratio of 1:50 (w/w) and approximate sample concentration of 0.2 % in Tris buffer (33). The reaction was ended by freezing. The digested samples (20ml) were applied directly to the HPLC system, consisting of Shim-pack HRC-C8, 25X4.6 mm reverse phase column and detected by an UV detector, SPD-6A, l: 275 nm. as described by Sandberg et.al. (35,36), the major thermolytic peptide, tyrosyl-glycine (YG) with an elution time of 12.6 min., was used for elastin quantitation as compared to its commercial standard (Sigmaã). The results were calculated using a conversion factor (1.87 x nMoles YG = mg elastin) as reported by Blankenship et al (37). The results were expressed as mg/g wet tissue.

Statistical Evaluation: The significance of the difference observed between the control and experimental groups for the parameters; serum Cu levels and Cp activities were evaluated using Student-t test, lung and body weight, DES, IDES and elastin contents of the tissues were evaluated by Mann-Whitney U test.

Figure 1. The physical appearance of the control (C) and experimental (E) newborn on the 7th day.



It can be noted that the newborn from the DPA-treated group shows significant reduction in weight, wrinkled and lax skin compared with the newborn from the control group.

RESULTS

We observed marked changes in skin elasticity on DPA treated group newborn rats, compared with those of the control group (Figure 1). At the end of the experimental period, the lung and body weight measurement showed significant growth retardation and loss of body and lung weight (p=0. 001) (Table I). In order to investigate the effects of DPA on maternal and newborn groups, serum Cu and Cp levels were

assessed. These results showed that serum Cu and Cp levels were reduced in the DPA treated groups ($p<0.001$) (Table II). DES, IDES and elastin contents of experimental newborn lung tissues were compared with those of the control group. It was found that DES, IDES and elastin contents were significantly decreased in the DPA treated groups' offspring (p=0. 0004, p=0. 002, p=0. 05) (Table III).

Table I. Effect of DPA treatment on lung and body weight of the newborn

Newborn (21st day)	N	Lung weight (g)	Body weight (g)	Lung/Body weight (%)
Control group	10	0.490±0.066	48.64±4.58	1
Experimental group	10	0.243±0.026*	26.82±3.64*	0.9

N= total number of animals in a group;
Results are mean ± SD of N animals
**Significantly different from the control group (p: 0.001) by Mann-Whitney U test.*

Table II. Serum Copper (Cu) and Ceruloplasmin (Cp) levels of control and experimental groups

Parameter	N	$Maternal_C$	$Maternal_E$	$Newborn_C$	$Newborn_E$
Serum Cu (μg/dl)	20	64.38±7.38	24.33±3.05*	55.44±4.56	11.70±1.75*
Cp (U/L)	20	54.05±4.81	16.31±2.96*	22.96±2.26	3.50±0.76*

Table III. Elastin, desmosine and isodesmosine contents and desmosine/elastin and isodesmosine /elastin ratio of control and experimental newborn lung tissues

Parameter (μg/g wet tissue)	N	$Newborn_C$	$Newborn_E$	Reduction ratio (E/C)(%)
Elastin	10	170.24±29.92	90.57±24.62***	47
Desmosine	9	24.78±12.81	10.90±3.83**	56
Isodesmosine	9	7.16±1.50	4.90±1.58*	72
DES/Elastin		0.14	0.12*	
IDES/Elastin		0.042	0.054*	

N= total number of animals in a group;
Results are mean ± SD of N animals
$_C$: Control group,
$_E$: Experimental group,
*E/C: Per cent reduction ratio between experimental (E) and control (C) group parameters ***(p=0.0004), ** (p=0.002), *(p=0.05), significantly different from the control group by Mann- Whitney U test.*

DISCUSSION

D-Penicillamine is not immediately toxic for cells; however, it has been shown to affect transaminase and deaminase activities, to decrease the concentration of Cu and other cations and vitamin B6(38,6), and to impair the formation of intermolecular cross-links in collagen and elastin (39). In the rat, lung growth and functional maturation proceed through several well-characterised stages and are almost complete at the age of 40d. Lung elastin content raises slowly from d4 to 12, increases 3 fold between d12 to 20 with equilibrated lung growth occurring between 20-40d. Likewise, elastin cross-linking (DES and IDES)increases by 10-12 fold and reaches adult values by day 20-21 (40,41).

In our experimental conditions, the complexity of the effects resulted in the inhibition of the offspring growth (Figure 1). Therefore, although we did not observe a considerable decrease of the lung weight/ body weight ratio in the experimental group, the molecular composition of the lung and the quality of the essential elastic component, elastin, have significantly changed as will be discussed further below. Although DPA (400mg/kg) was injected to maternal rats in the perinatal period, all newborn rats suffered from DPA induced Cu depletion in both the gestational and the suckling periods. Cu depletion effects of DPA were observed also significantly with serum Cu and Cp levels in maternal and newborn groups ($p<0.001$). Animal and human studies have shown that copper is required for infant growth, host defence mechanism, bone strength, red and white cell maturation, iron transport, cholesterol and glucose metabolism, myocardial contractility and brain development (27,42). Copper deficiency in the perinatal period produces a variety of clinical effects, which is not surprising in view of its known roles in the structure and function of a number of enzymes including lysyl oxidase, which is related with elastin maturation in the connective tissue (43). On

the other hand DPA is a drug causing B6 (pyridoxine) deficiency, and Myers et al. (44) have shown that B6 deficiency caused cross-linking impairment on lung elastin in perinatal and weaning rat pups. The objective of our investigation was to observe whether copper depletion might reduce the insoluble elastin content and the intermolecular cross-linking in the perinatal period extending from the third trimester to the 21st day of the suckling period. This period is interesting due to the fact that it is the most important period for the formation of elastin cross-links and also for copper accumulation in the fetus during the third trimester. During the post-natal period, the only means of copper obtaining for the infant is the suckling.

The results showed that elastin, DES and IDES contents of newborn rats were significantly reduced by copper depletion in the perinatal period (Table III). This reduction seemed to be concomitant with the deficiency of Cu in the maternal and newborn groups. In addition to this quantitative data, we also tried to address the possible specific difference in the ratios of the intramolecular cross-links (mg DES/ mg Elastin and mg IDES/ mg Elastin). We observed a significant decrease in DES ratio for the experimental group compared with the control while the IDES ratio showed a slight increase (p=0. 05). The comparison of our data on the reduction (47% for elastin, 56% for DES and 72% for IDES) with those of Dubich et al. (18) indicate that the reduction observed in our study for elastin, DES and IDES levels are more pronounced than that observed by Dubich et al. In addition, their method could not separate DES and IDES. This discrepancy in the reduction observed can be interpreted in terms of the dosage used: we administered a dosage of 400 mg/kg intraperitoneally while Dubich et al. applied a concentration of %0.2 in the food given to the rats, which may fluctuate due to the amount of food taken in by the rats daily. In addition, the experimental set-up and the model used by Dubich et al. are significantly different than ours: mainly, the dosages of drug used, isolation and detection methods of elastin, isodesmosine and desmosine, respectively. Cross-link formation results in the complete insolubilization of soluble precursor molecules of elastin; therefore, inhibition of lysyl oxidase (E.C 1.4.3.13) by eliminating Cu resulted in an accumulation of soluble elastin, due to the defect in cross-linkage. Although not 100% effective, copper deficiency does allow for the accumulation of significant amounts of soluble elastin (45). Our findings are in agreement with the abnormalities found in Menkes and occipital horn syndromes from the point of view of cross-linking (21). These abnormalities are associated with a reduction in the activity of lysyl oxidase, an extracellular Cu enzyme that initiates the cross-linking of elastin and collagen. Gacheru et al. (46) have suggested that low levels of lysyl oxidase activity in Menkes and occipital horn syndromes may occur secondarily and consequently impair incorporation of Cu into lysyl oxidase. On the other hand, Cp is responsible for the transport of approximately 95% of the Cu in serum but the mechanism for intracellular Cu transport are not clear yet. Rucker et. al. (47) have shown that Cu deficiency does not influence the steady state levels of lysyl oxidase specific mRNA in rat skin and therefore the activity of lysyl oxidase may be related with Cu incorporation into the enzyme protein. Relating to our data, the working hypothesis could be that DPA affects the gestational and postnatal maturation of lung elastin. Although we couldn't assess the soluble elastin content, we observed highly significant decrease in the insoluble elastin and DES/Elastin ratio, so our proposal is that, it should be related to change crosslink formation and Cu depletion in the perinatal period. Our experimental model includes both gestational and post-natal periods and this model may be useful for working on elastin metabolism and maturation during the perinatal period in rats

In conclusion; the results of the present study show that DPA induces a change in the cross-linking in lung elastin, DES and IDES during the perinatal period. Its inhibitory effects on cross-linking include

a more complex mechanism than merely copper chelation, as suggested by previous reports. The main observation from this study is that DPA did affect perinatal lung biochemistry even though it was not administered directly to the pups.

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mg/kg/day) was administered to 20 maternal rats intraperitoneally (i.p) beginning on the 14th day of gestation until ending on the postnatal 21st day during the all-suckling period. In the same period physiologic saline was administered to 20 maternal rats to obtain control group. At the end of the experimental period, 20 newborn rats were selected randomly from each breeder, 10 from control and 10 from experimental group. The body and lung weights of newborns were assessed. Serum copper (Cu) levels and ceruloplasmin (Cp) activities of the maternal and newborn groups and lung tissue elastin, desmosine (DES) and isodesmosine (IDES) levels in the newborn groups were measured with comparing with the control groups. Atomic absorption spectrophotometer and spectrophotometric methods were used for Cu and Cp measurements respectively. High Performance Liquid Chromatography (HPLC) was used for the determination of DES and IDES, the specific cross-linking amino acids in elastin, and elastin levels. The results showed that DPA induced copper depletion caused loss of skin elasticity and reduction in body and lung weight in experimental newborns group. The serum Cu levels and Cp activity in maternal and newborn experimental groups were found 24.33±3.05mg/dl, 16.31±2.96 U/L and 11.70±1.75mg/dl, 3.50±0.76U/L respectively. In the maternal and newborn of control groups Cu and Cp values were 64.38±7.38mg/dl, 54.05±4.81U/L, 55.44±4.56mg/dl, 22.96±2.26U/L respectively. The lung DES, IDES and elastin values of control were 24.78±12.81mg/g, 7.16±1.50mg/g, 170±29.92mg/g wet-tissue and in experimental groups were 10.90±3.83mg/g, 4.90±1.58mg/g, 90.57±24.62mg/g wet-tissue respectively. The statistical evaluations of data were made using Student-t and Mann-Whitney U tests according to the number of groups. We found significant difference for Cu levels and Cp activities between control and experimental groups ($p<0.001$). DES, IDES and elastin levels showed significant decreases in experimental newborn group compared with the control group ($p=0.0004$, $p=0.0020$, $p=0.05$). In conclusion; our experimental model includes both gestational and postnatal periods and this model may be useful for working with the effect of DPA induced copper depletion on the maturation of the newborn connective tissue. Another conclusion drawn from this study is that Cu depletion in middling quality due to DPA administration induces change in cross-linking in lung elastin during the perinatal period.

Key Words: *Desmosine, D-Penicillamine, Elastin, Isodesmosine, Perinatal period.*

INTRODUCTION

D-Penicillamine (dimethylcysteine) is a degradation product of penicillin and is prepared by its hydrolysis. Only the D isomer of penicillamine is recommended for clinical application and is used mainly in the therapy of Wilson's disease (1-3), cystinuria (4) and rheumatoid arthritis (5). DPA is also an effective chelator of copper (Cu), mercury, zinc and lead, promoting the excretion of these metals in urine (6). The wide use of DPA in human therapy and the reports in the literature associated with pathological events have stimulated a series of experimental studies on the effect of this drug on various organs and systems (7 - 12). Elastin is a rubber-like insoluble protein presents in mammalian connective tissue (13,14) mostly, ligaments, blood vessels and lung. The extreme insolubility of elastin is due to the presence of covalent cross-links between groups on the side chains of lysine residues, which lie in proximity to adjacent polypeptide chains (15). DPA-treated weaning rats ave been demonstrated to contain less mature elastin than normal rats (16-18).The effect of DPA on elastin cross-linking is associated with its being a chelating drug and Cu being an important cofactor for lysyl oxidase (19). Desmosine (DES) and Isodesmosine (IDES) are specific cross-linking amino acids and isomers formed by the condensation of three-aminoadipic acid d-semialdehyde (allysine) residues with one lysine residue in elastin (20) and a number of investigations related to the cross-linking in various pathologies have been published in literature (21 - 23). Elastic and collagen fibers confer recoil and tensile strength to the pulmonary vasculature, airways, alveolar walls, and pleura. These durable extracellular matrix components are primarily synthesised during lung development and growth, and are expressed at very low levels in adult rat lung (24). DPA is the most commonly long-term used drug in some metabolic diseases such as Wilson, scleroderma (25), and rheumatoid arthritis, its usage in pregnancy is a controversial issue because of an association with a generalised connective tis-