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In-Vitro Effect of Phthalate Esters on Retinal Aldolase

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Background and Aim: Phthalate esters used in the manufacture of cosmetics, lubricants, medical devices and wood furnishings enter the human system through various routes and accumulate into tissues to a considerable level. Low levels of plasticizer cause infertility, hypospadias and premature puberty, while high doses of esters can damage the liver and lung. Reports on the ocular effects of these compounds are either not available or very scarce. The present study assessed the impact of dimethyl phthalate (DMP), diethyl-phthalate (DEP) and diethyl hexyl-phthalate (DEHP) on bovine retinal aldolase.

Materials and Methods: Retinal aldolase activity of 0.1 ml of 1:10 solution of dialyzed retinal homogenate was assayed at 540 nm. The aldolase activity of the retinal homogenate after incubation in the above-mentioned tubes of DMP, DEP and DEHP was similarly assayed.

Results: In the present study, the aldolase activity of the retina was significantly inhibited by phthalate esters and was directly proportional to the molecular size of esters. When comparing the sum of the mean values of the tested esters, the inhibition of aldolase activity increased in the following order - DEHP < DMP < DEP.

Conclusion: Based on our results, we suggest that exposure to phthalate esters should be avoided in order to reduce the risk of ocular damage.

Key Words: DMP, DEP, DEHP, phthalate ester, retina

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Fitalat Esterlerin Retinal Aldolaz Üzerine İn Vitro Etkileri

Giriş: Kozmetikler, kaydırıcılar, tıbbi araçlar ve ağaç parlaticıların yapısında kullanılan fitalat esterler, insan vücuduna çeşitli yollarla girer ve dokularda birikirler. Yumuşaticıların düşük dozları bile infertilite, hipospadias ve prematüre doğumlara yol açabilirken, esterlerin yüksek dozları karaciğer ve akciğer hasarına yol açabilir. Bu bileşiklerin göz üzerine olan etkileri ile ilgili yayınlar yetersizdir.

Amaç: Bu çalışmada dimetil fitalat (DMP), dietil-fitalat (DEP) ve dietil heksil fitalat (DEHP)'in sıgır retinal aldolaz düzeylerine etkisi araştırılmıştır.

Yöntem ve Gereç: 0.1 ml, 1/10'luk diyalize edilmiş retinal homojenat'ın retinal aldolaz aktivitesi 540 nm'de ölçüldü. Retinal homojenatın DMP, DEP ve DEHP içeren tüplerde inkübasyonunu takiben ölçümler tekrarlandı.

Bulgular: Retinanın aldolaz aktivitesi fitalat esterleri ile anlamlı şekilde inhibe edildi ve bu inhibisyon direk olarak esterin moleküler büyüklüğü ile ilişkiliydi. Test edilen esterler karşılaştırıldığında aldolaz aktivitesinin inhibisyonu şu sırayla artış gösterdi: DEHP < DMP < DEP.

Sonuç: Bu bulgular ışığında oküler hasar riskini azaltmak için fitalat esterlere maruz kalmaktan kaçınılması gerektiği görüşündeyiz.

Anahtar Sözcükler: DMP, DEP, DEHP, fitalat ester, retina

Introduction

Phthalate esters are dialkyl or alkyl aryl esters of 1,2 benzene dicarboxylic acid. These are widely used to impart flexibility to otherwise rigid polyvinyl chloride (plastics). They are also utilized in the manufacture of perfumes, hair sprays, lubricants, wood furnishings and medical devices, e.g. blood bags, tubing, surgical gloves, dialysis tubing, and nasogastric tubing (1). These substances can, however, enter the human system through ingestion, inhalation, skin and parenteral routes. In humans, low doses of plasticizers have been reported to cause infertility hypospadias (2), and consequently damage the Sertoli cells leading to damaged sperm (3-5) in males and premature puberty in females (6-8). In contrast, high doses of these plasticizers in experimental animals can cause liver (9) and lung damage (10), including liver cancers (11,12). Unfortunately, no reports are available regarding the ocular effects of these compounds.

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In this premier study we report the in-vitro inhibitory effect of dimethyl phthalate (DMP), diethyl phthalate (DEP) and diethyl hexyl phthalate (DEHP) on bovine retinal aldolase activity. The retinal aldolase is an important glycolysis enzyme and plays a vital role in formation of Schiff's protonated bases in the visual cycle. The defect may, therefore, be anticipated to cause accumulation of toxic byproducts of the visual cycle in retinal cells resulting in widespread chromatophore damage.

Materials and Methods

Freshly enucleated buffalo eyes were obtained from the slaughterhouse within 30 min of sacrifice of animals. The retina was dissected out immediately. The isolated retinal tissue was homogenized at 4 °C in Teflon homogenizer for 10 min to obtain a smooth, uniform homogenate. The homogenate was centrifuged at 5000 rpm for 20 min at 4 °C in refrigerated centrifuge. The supernatant fluid of centrifuged retinal homogenate was dialyzed against double-distilled water at 4 °C for 18 h with three changes of water at 6 h intervals. 0.01 ml, 0.5 ml, 0.1 ml, and 0.5 ml, 1.0 ml and 0.12 ml of 0.125M methanolic solution of DMP, DEP and DEHP was transferred to separate tubes consecutively and the final volume was made to 1 ml by absolute methanol. Methanol was evaporated by incubating these tubes in

boiling water bath for 30 min. Retinal aldolase activity of 0.1 ml of 1:10 solution of dialyzed retinal homogenate was assayed as described by Sibley and Lehninger (13) at 540 nm. The aldolase activity of the retinal homogenate after incubation in the above-mentioned tubes of DMP, DEP and DEHP was similarly assayed.

Results

The inhibitory effect of DMP on the aldolase activity of the retina varied considerably and was concentration-dependent (Table 1). Generally, the aldolase activity was higher at lower doses of DMP, and decreased consistently with an increase in DMP concentrations. A maximum aldolase activity (98.76%) was observed at 1.25 mM of DMP, and this decreased to 43.3% at 125 mM of DMP. Thus, from the data, a maximum inhibition (56.7%) was determined at 125 mM of DMP, which was followed by a 44% inhibition in aldolase activity at 62.5 mM of DMP. The slope of DMP inhibition was $\tan^{-1} 1.39$ up to a concentration of 62.5 mM. Similarly, DEP also adversely affected aldolase activity of the retina (Table 2). However, with DEP, maximum aldolase activity (100%) was observed at the lower dose (1.25 mM) compared with that observed at the same concentrations of either DMP (98.76%) or DEHP (87.92%). The decline in the aldolase activity in general, however, was greater than that observed for DMP at all concentrations, the maximum

Table 1. Effect of DMP on the aldolase activity of retina.

| Tubes of Incubation | Normal | 1 | 2 | 3 | 4 | 5 |
|--------------------------------------|--------|-------|-------|-------|-------|-------|
| Concentration of DMP used (μ M) | 0.0 | 1.25 | 6.25 | 12.5 | 62.5 | 125 |
| Optical density (λ 540 nm) | 0.323 | 0.319 | 0.305 | 0.292 | 0.181 | 0.140 |
| Percent activity | 100 | 98.76 | 94.40 | 90.40 | 56.00 | 43.30 |
| Percent inhibition | 0.0 | 1.24 | 5.60 | 9.60 | 44.00 | 56.70 |

Table 2. Effect of DEP on the aldolase activity of retina.

| Tubes of Incubation | Normal | 1 | 2 | 3 | 4 | 5 |
|--------------------------------------|--------|-------|-------|-------|-------|-------|
| Concentration of DEP used (μ M) | 0.0 | 1.25 | 6.25 | 12.5 | 62.5 | 125 |
| Optical density (λ 540 nm) | 0.323 | 0.323 | 0.268 | 0.208 | 0.184 | 0.125 |
| Percent activity | 100 | 100 | 82.97 | 64.39 | 56.96 | 38.70 |
| Percent inhibition | 0.0 | 0.0 | 17.03 | 35.61 | 43.04 | 61.30 |

Table 3. Effect of DEHP on the aldolase activity of retina.

| Tubes of Incubation | Normal | 1 | 2 | 3 | 4 | 5 |
|--|--------|-------|-------|-------|-------|-------|
| Concentration of DEHP used (μM) | 0.0 | 1.25 | 6.25 | 12.5 | 62.5 | 125 |
| Optical density ($\lambda 540 \text{ nm}$) | 0.323 | 0.284 | 0.280 | 0.276 | 0.260 | 0.260 |
| Percent activity | 100 | 87.92 | 86.68 | 85.44 | 80.49 | 80.49 |
| Percent inhibition | 0.0 | 12.08 | 13.32 | 14.56 | 19.51 | 19.51 |

being 38.7% aldolase activity at 125 mM. The inhibition in aldolase activity expressed in terms of percent inhibition followed a trend similar to that observed for DMP. The slope of DEP inhibition curve was $\text{Tan} - 4.0$ up to 12.5 mM concentration. Furthermore, when DEHP was tested, it was found as a weak inhibitor of aldolase activity compared to DMP and DEP, at all the tested concentrations. Interestingly, the aldolase activity following the highest concentration (125 mM) of DEHP (80.49%) did not change significantly compared to that observed at 12.5 mM (85.44%) of DEHP. In comparison, the aldolase activity was nearly identical at 1.25 mM (87.92%) and 6.25 mM (86.68%) of DEHP (Table 3). However, the aldolase activity decreased progressively at all the tested doses of DEHP. Indeed, among all three esters and the concentrations used, DEP exhibited greater inhibition in aldolase activity at all concentrations except at 62.5 mM of DMP, which showed greater inhibition (44%) compared to DEP (43.04%) and DHEP (19.51%). Thus, a comparison of the sum of mean values of the five concentrations (excluding normal dose) of the three esters, the percent inhibition of retinal aldolase activity increased in the following order - DEHP < DMP < DEP. The inhibition slope was $\text{Tan} - 13.0$ up to a concentration of 1.25 mM, which is maximum compared to that of DMP and DEP.

Discussion

In the present study, the three phthalate esters were found inhibitory to retinal aldolase. The rate of inhibition

of the enzyme was found to be linear up to a concentration of 62.5 mM of DMP, 12.5 mM of DEP and 1.25 mM of DEHP. The amount of the esters effective on the rate of inhibition of retinal aldolase decreased with an increase in the molecular size of the ester. In comparison, the slope of inhibition curve increased with an increase in the molecular size. This study thus clearly indicated that the potency of inhibition is proportional to the molecular size of the phthalate esters used. The decrease in the effective quantity of the esters on inhibition of the enzyme activity with the molecular size of the ester may be due to the slow rate of solubility of esters, which increases with an increase in their molecular size. In a similar study, the solubility of the phthalate ester in aqueous medium has been reported to decrease with an increase in the molecular size of the phthalate esters (10). Inhibition of retinal aldolase, a key enzyme of energy metabolism, as observed in this study is a significant observation since phthalate esters may play an important role in the carbohydrate metabolism of the retina. Under conditions of intact blood retinal barrier (BRB), no studies are available to suggest that the phthalate esters cross the BRB. However, it may be speculated that low molecular weight plasticizers like dimethyl phthalate ester may penetrate the BRB. Further in vivo studies are required, however, to ascertain the exact damage caused by the plasticizers to the living retina. In conclusion, the present study suggests that exposure to phthalate esters should be avoided in order to reduce the risk of ocular damage.

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