Decreased of Clusterin mRNA Expression of Epididymis Following Exposure to Bisphenol A Diglycidyl Ether during Gestation and Lactation in Sprague-Dawley Rats

Dae-woong Kang, Su-kyoung Kwon, Yun-jung Yang, Young-jin Chun and Yeon-pyo Hong

Department of Preventive Medicine, College of Medicine, Chung-Ang University, Seoul 156-756, Korea
1College of Pharmacy, Chung-Ang University, Seoul 156-756, Korea

임신 및 수유기간 동안 bisphenol A diglycidyl ether 노출에 의한 란트 부고환 clusterin mRNA 발현량 감소

강대웅, 권수경, 양윤정, 전영진, 홍연표

중앙대학교 의과대학 예방의학교실, 1중앙대학교 약학대학

요 약

Bisphenol A diglycidyl ether (BADGE)는 비스페놀 A와 에피클로로화이드인의 축합에 의해 만들어지는 물질로 상업용 핵상 예폭시 수지의 주성분이다. 본 연구는 clusterin mRNA 발현이 BADGE의 노출된 생식기계 독성에 연관되어 있는지를 연구하기 위해 수행하였다. BADGE는 SPF Sprague-Dawley 임신 란트 에 임신 6일부터 수유기까지 하루에 한 번 0(대조군)과 375 mg/kg/day를 경구 투여하였다. 수컷 세끼는 일반 사향과 품목, 일반 발달 지표, 이개개진, 몸무게, 혈액 등의 차이가 현저한 차이를 보이지 않았다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6와 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다. BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다..BADGE 375 mg/kg/day 투여군에서 맥파와 생식기 사이의 멀리 떨어진 후 3, 6과 9주에 붙임하여 부고환의 조직학적 변화 등을 관찰하였다.

Key words: bisphenol A diglycidyl ether, clusterin expression, development, rat epididymis, gestation and lactation period

INTRODUCTION

Bisphenol A diglycidyl ether (BADGE) is a commercial substitute for bisphenol A (BPA). BADGE is an epoxy resin monomer that is obtained by a conden-
sation reaction between epichlorohydrin and BPA (IARC, 1999). The potency of estrogenic activity by BADGE is less than 1/100 that of BPA (WWF, 2000). BPA (starting agent of BADGE), already known as an endocrine disruptor (WWF, 2000), showed abnormalities of the reproductive tract in experimental animals and could act at very low doses in the range of human and wildlife environmental exposures (vom Saal et al., 1997; Sheehan et al., 1999). Occupational exposure to BADGE occurs in its epoxy resin production and in various uses of epoxy products (IARC, 1999). Epoxy resins based on BADGE are strong contact sensitizers (Thorgerisson and Fregert, 1977; Thorgerisson et al., 1978) and are among the most common causes of occupational allergic contact dermatitis (Jolanki et al., 1987; Kanerva et al., 1988; Jolanki et al., 1990).

Clusterin is a ubiquitously expressed amphipathic glycoprotein that consists of two non-identical subunits designated \( \alpha \) and \( \beta \). The 34-kDa \( \alpha \) and 47-kDa \( \beta \) subunits of rat clusterin are held together by a disulfide bond (Kissinger et al., 1982) and are expressed in a variety of tissues in response to cellular stress (Savkovic et al., 2004). The earliest studies of clusterin were performed in the reproductive system where clusterin was identified as a major protein in the secretory products of cultured rat Sertoli cells and in the fluid of ram rete testis (Kissinger et al., 1982; Blaschuk et al., 1983). Clusterin is the first well-characterized, constitutively secreted extracellular chaperone, which binds to exposed hydrophobic regions on non-native proteins (Lee et al., 2000). In many hormone-dependent tissues, such as prostate and mammary gland, clusterin expression is induced after hormone ablation and appears to be involved in the apoptotic processes associated with tissue remodeling (Sylvester et al., 1991). Clusterin has been revealed to be involved in a variety of physiological processes important for carcinogenesis including apoptotic cell death, cell cycle regulation, DNA repair, cell adhesion, tissue remodelling, lipid transportation, membrane recycling, as well as immune system and complement regulation (Wilson and Easterbrook-Smith, 2000; Pucci et al., 2004; Trougakos et al., 2004).

Northern analysis and in situ hybridization revealed that clusterin mRNA was in highest abundance in epididymal cells (Tenniswood et al., 1992).

The epididymis is one of the reproductive organs which is a long, single convoluted tubule through which spermatozoa must transit to acquire progressive motility and fertilizing ability (Thimon et al., 2007), and to create the luminal microenvironment necessary for maturation, storage and survival of spermatozoa (Hinton and Palladino, 1995; Hinton et al., 1996).

There was no evidence of reproductive or endocrine toxicity, the upper ranges of dosing being determined by maternal toxicity in one- and two-generation reproduction studies and developmental investigations on BADGE (Poole et al., 2004). However, there is some evidence of estrogenic effects from in vitro tests (Olea et al., 1996; Perez et al., 1998). Recently, acute (Im et al., 2006) and developmental studies (Hyung et al., 2007) of oral exposure to BADGE showed reproductive and/or endocrine toxicity. Nevertheless, the mechanism of reproductive toxicity of BADGE has not been studied yet.

This study was performed to determine whether clusterin mRNA expression in rat epididymis is involved in the reproductive effects of BADGE exposure.

**METHODS**

1. Animals

This experiment was performed in accordance with the Good Laboratory Practice (GLP) guidelines for Animal Experiments of Chemon Co. Ltd. Eight-week-old SPF (specific pathogen-free). Sprague-Dawley rats were purchased from the Charles River breeding laboratory (Wilmington, Mass, USA). The dosing solutions were prepared by thoroughly mixing BADGE in corn oil at the proper concentrations (Table 1). BADGE was administered once daily by gastric gavage to pregnant 9-week-old SPF Sprague-Dawley female rats from gestation day 6 to lactation at doses of 0
(control) and 375 mg/kg/day. The dose of BADGE used was based on previous results showing that in gestation and lactation exposure to this dose observed change of body weight and relative weight of epididymis (Hyung et al., 2007). Dosing volumes were 10 mL/kg, and doses were based on the most recent individual body weights.

The litters were culled on postnatal (PND) 4 to 3 male pups per dam. Fifteen pups were allocated in control and each treatment group. Only 1 dam was delivered 3 pups. All allocations were performed by computerized random selection. After lactation period, male pups were fed rodent ration (Purina Korea Inc, Gyeonggi, Korea). Five male pups was sacrificed by ether at control and each treatment groups in 3, 6, and 9 weeks (Fig. 1). The epididymis was excised and weighted, then stored at −80°C until analysis.

2. Observed items

1) Body and epididymis weight

Female dams had their body weight measured on gestational day(GD) 0, 6, 9, 12, 15, and 20 as well as on PND 0, 7, 14, and 21. Male pups had their body weight measured on PND 4, 7, 14, 21, 28, 35, 42, 49, 56 and 63. The weight of the epididymis was measured on PND 21, 42, and 63. Relative epididymis weight (epididymis weight/body weight × 100) were compared control with treatment group.

2) Observations of pups development

Each pup was weighed and received a detailed physical examination on PND 1, 4, 7, 14 and 21 and weekly thereafter. Anogenital distance (AGD) was measured on PND 0, 4, 7, 14, and 21. Pina detachment was measured on PND 2, 3, and 4. Incisor eruption was measured on PND 8 to after. AGD was adjusted by body weight. Thoracic nipple retention was evaluated for all male pups on PND 11 to 14. Eye opening was measured on PND 12 and thereafter. Testis descent was measured on PND 16 to 22.

3) Histology of epididymis

For each group necropsy and selective histological examination were conducted on PND 21, 42 and 63. The epididymis was preserved in Bouin’s solution for approximately 24 hour and afterwards washed several times with ethanol (70%) before embedding in paraffin. Embedded tissue was sectioned at 4 μm and tissue sections were stained with PAS and hematoxylin-eosin (Leblond and Clermont, 1952) and examined under a light microscope (Olympus, Japan).

3. Real time PCR for mRNA expression of clusterin

Clusterin mRNA was analyzed by the quantitative real-time polymerase chain reaction (PCR) method. Total RNA was isolated from cauda epididymis using RNeasy mini kit (Qiagen, Hilden, Germany). The reverse transcription was carried out from 3 μg total RNA using a first strand cDNA synthesis RT-PCR (AMV) kit (Roche Applied Science, Mannheim,
Table 2. Clusterin oligonucleotides used in this study

<table>
<thead>
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<th>Oligonucleotide</th>
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<th>Pair quality</th>
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<td>22</td>
<td>60.7</td>
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<tr>
<td>CCTGAGGCGGTCTGGAATCTC</td>
<td></td>
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</table>

(*: amplicon melting temperature, **: optimal annealing temperature) program design: Beacon designer

Germany) using 10× RT-PCR buffer, 1 mM dNTP, 50 U RNase inhibitor, ≥20 U Avian Myeloblastosis Virus(AMV) reverse transcriptase and 100 pM of oligo-dT primer. The cDNA synthesis was performed at 25°C for 10 min, 42°C for 1 hr, 94°C for 10 min, and then by rapid freezing at 4°C.

Clusterin was amplified from cDNA with a ratspecific primer (Table 2). The selected PCR bands were prepared by cloning sequences and amplified by these primers into the plasmid pCR® II-TOPO® (Invitrogen, Karlsruhe, Germany), and cloned sequences were verified by sequence analysis. Determined DNA sequences were analyzed by searching for similarities using a BLAST program of National Center for Biotechnology Information GenBank. Real time PCR reactions were performed in triplicate using identical samples of epididymal total RNA. Twelve nanogram aliquots of cDNA synthesized from total RNA were added to a prepared PCR mixture containing 2X SYBR® Green I kit (Qiagen, Valencia, CA, USA) and each forward and reverse primers for clusterin. Real time PCR reactions cycle were consisted of the following: on cycle of denaturing at 95°C for 10 minutes, followed by 45 ~ 50 cycles of denaturation at 94°C for 10 seconds, annealing at 62°C for 20 seconds, extension at 72°C for 20 seconds, and the final extension at 72°C for 5 seconds and 30°C for 10 minutes. The amplified clusterin PCR products were normalized to rat GAPDH.

4. Statistical analysis

Data are expressed as means and standard deviation. Statistical analyses were performed using Kruskal-Wallis test. The criterion of significance was set at p<0.05.

Fig. 2. The changes of body weight by age in SD male pups exposed to BADGE. All values are means and standard deviation. Statistical analyses were performed using Kruskal-Wallis test. Body weight of male pups at dose levels of 375 mg/kg/day were lower than control group at PND 21, 35, 42, 56 and 63 (+: p<0.05, **: p<0.01).

RESULTS

1. Changes of body and epididymis weight

In BADGE 375 mg/kg/day treated male pups, there was no statically difference observed until PND 14 compared to control. After PND 21, body weight gain in BADGE 375 mg/kg/day treated male pups was observed lower than the control at PND 21, 35, 42, 56 and 63 (p<0.05) (Fig. 2). The relative weight of epididymis in control and BADGE treated male
pups was 0.066±0.018 and 0.064±0.011, 0.113±0.013 and 0.133±0.028, and 0.179±0.009 and 0.185±0.013 at PND 21, 42, and 63, respectively. There was no statistical differences between control and BADGE treated group (Fig. 3).

2. Developmental changes of pups

General development items which were separation of auricles, eruption of incisor, separation of eyelid, nipple retention, decendent of testis, and separation of prepuce- in the treatment group showed no difference from the control (data was not shown). Adjusted AGD (AGD/BW, mm/g) in BADGE treated male pups were longer than control group at PND 7 and 14 (p<0.05) (Fig. 4).

3. Histology of epididymis

Histological observation of epididymis was done to investigate the adverse effects of developmental exposure to BADGE on male pups epididymis (Fig. 5). Observation items were including blood vessel, cilia, connective tissue, stereocilia, smooth muscle, and epithelium. The histological findings in epididy-

4. The relative clusterin mRNA expression

Male pups were exposed to BADGE during gestation and lactation, and the clusterin mRNA expression of epididymis in male pups were measured by real-time PCR. Relative mRNA expression of treatment group which were 0.439±0.132 (p<0.05) in 3-week-old rats, 0.434±0.312 (p<0.05) in 6-week-old rats, and 0.145±0.075 (p<0.01) in 9-week-old rats were statistically lower than control group (Fig. 6). Especially, in 9-week-old rats, clusterin mRNA expression of epididymis in treatment group was suppressed to 86% of control.

DISCUSSION

We performed this experiment to know that clusterin was involved in epididymal effect of BADGE during the time of windows of pregnancy and lacta-
Fig. 5. Histological findings in epididymis (H & E staining, ×100). No histological changes were found in epididymis (BV: Blood vessel, C: cilia, CT: Connective tissue, SC: Stereocilia, SM: Smooth muscle, EP: Epithelium).

tion. Clusterin mRNA expression in the epididymis was down-regulated by BADGE exposure during gestation and lactation; expression was reduced by more than 57% in the prepubertal period and 86% in the adult period. This results indicated that the effect of BADGE on epididymis was more sensitive in the
clusterin at low/moderate level in human serum reported cytoprotective effects (Trougakos et al., 2005). Clusterin have activity in cell-cell adhesion and cellular differentiation (Thomas-Salgar and Millis, 1994). Therefore, down regulation of clusterin mRNA expression in the epididymis suggested that the effect of BADGE on epididymis was decreased activity of cell differentiation and role of molecular chaperon.

Body weight, for male pups treated with doses of 375 mg/kg/day, was significantly lower than in the control group at PND 21, 35, 42, 56 and 63 (P < 0.05). This result was similar to the results of Hanley et al. (1996) where decreased body weight in male offspring of treated rats, at dose levels of 540 and 750 mg/kg/day, was observed in a two-generation reproduction study. Another study showed that while treatment at all dose levels had no adverse effects on reproductive performance, a slight reduction in mean pup weight in the 540 mg/kg group was observed on day 21 (Smith et al., 1989). In other sub-chronic dietary studies, rats receiving the highest dose levels (i.e., 4,500 mg/kg/day) rejected the diet and failed to gain weight (Wolf, 1958). Adjusted AGD in BADGE 375 mg/kg/day treated pups were showed longer than control group at PND 7 (p < 0.05) and PND 14 (p < 0.01). AGD, an external marker of sexual differentiation at birth, is normally regulated by testosterone from the fetal testis and can be used as a marker of fetal exposure to endogenous fetal and transplacentally acquired maternal androgens (Graham and Gandelman, 1986) and increased in male pups with maternal low protein diets during pregnancy and lactation in the rat (Zambrano et al., 2005). Therefore, this finding might be related increased maternal androgen. Further studies on the digestive effects of BADGE are needed.

This results suggested that epididymal toxicity of BADGE can be related to clusterin suppression. Further quantitative confirmation of expression changes by means of Western blotting analyses and determination of apoptotic changes, ultra-structural changes, dose-responses, and hormonal changes are required.

Fig. 6. The clusterin mRNA expression level of male pups in 3, 6, and 9 weeks exposure to BADGE during pregnancy and lactation period. Total RNA was isolated from epididymis of untreated or BADGE-treated (375 mg/kg/day) male rats. Expression level of clusterin mRNA was determined by real time RT-PCR and normalized with GAPDH level. The values represent the mean ± SD of three independent determinations. Statistical analyses were performed using Kruskal-Wallis test. In 3, 6 and 9 weeks, clusterin mRNA expression was significantly decreased to 56% (p < 0.05), 57% (p < 0.05), and 86% (p < 0.01) at BADGE treatment group, respectively (*: p < 0.05, **: p < 0.01).

sexually active period than in the prepubertal period. Clusterin, also known as testosterone-repressed message-2 (TRPM-2), sulphated glycoprotein-2 (SGP-2) and apolipoprotein J (ApoJ), was first isolated from the ram rete testis fluid (Blaschuk et al., 1983). Clusterin is the first well-characterized, constitutively secreted extracellular chaperone, which binds to exposed hydrophobic regions on non-native proteins. Chaperones may help control the folding state of extracellular proteins by targeting them for receptor-mediated endocytosis and intracellular lysosomal degradation (Lee et al., 2000). Molecular chaperones interact with various client proteins to assist in their folding and to enhance cellular recovery from stress conditions. Cellular stress and cell death are linked, as the induction of chaperones appears to function at key regulatory points in the control of apoptosis (Schwob et al., 1998). Extracellular and intracellular
CONCLUSION

Bisphenol A di glycidyl ether (BADGE) is the major component in commercial liquid epoxy resins. This study was performed to determine whether clusterin mRNA expression in rat epididymis is involved in the reproductive effects of BADGE exposure. BADGE was administered by gastric gavage to pregnant 8-week-old SPF Sprague-Dawley female rats from gestational day 6 to lactation once daily at doses of 0 (control) and 375 mg/kg/day. Five male pups in the control and treatment groups were culled and sacrificed by ether at PND 3, 6, and 9 weeks. The expression pattern of clusterin mRNA in the epididymis was observed by real-time PCR. The body weight gain of BADGE treated group tended to lower than control after PND 21. Adjusted AGD in BADGE treated pups at PND 7 and 14 was longer than in controls (p<0.05). Relative weights of epididymis were slightly heavier than in control in measures at PND 6 and 9 weeks. However, there were no significant changes in histological findings of epididymis between control and BADGE treated group. Clusterin mRNA expression of epididymis in the BADGE treatment group was significantly decreased by 56% at 3 weeks, 57% at 6 weeks, and 86% at 9 weeks compared to the control group. These results suggested that clusterin might be a BADGE-responsive gene that will predict potential reproductive effects in rat.

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REFERENCE

Blaschuk O, Burdzy K and Fritz IB. Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid, J Biol Chem 1983; 258: 7714-7720.


in dentistry, Environ Health Perspect 1996; 104: 298-305.


Schwochau GB, Nath KA and Rosenberg ME. Clusterin protects against oxidative stress in vitro through aggregative and nonaggregative properties, Kidney Int 1998; 53: 1647-1653.


Vom Saal FS, Timms BG, Montano MM, Palanza P, Thayer KA, Nagel SC, Dhar MD, Ganjam VK, Oarmeigiani S and Welshons WV. Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses, Proc Natl Acad Sci USA 1997; 94: 2056-2061.


