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서울대 약대 석사논문(2013년) 미국 정부 발간 SCI 학술지 '이달의 뉴스'로 선정

< 내용 및 의의 >

□ 서울대 약대 졸업생 권소연씨(28) 석사학위 논문(2013년 '중금속 납이 신장의 적혈구 탐식작용을 매개한 신장독성 유발기전에 관한 연구')이 미국 정부에서 발간하는「Environmental Health Perspectives」(EHP) 국제학술지에 2015년 2월호 논문게재와 더불어 '이달의 뉴스'로 선정되었다.

□ 이 학술지(EHP)는 이공계 분야 8,500 여개 SCI 학술지 중 유일하게 미국정부 (NIEHS; 미국 국립환경보건원)가 발행하는 매우 권위 있는 학술지이다. 선진국 과학자들조차 이 학술지에 게재하기가 어려운 현실에서, 석사학위 연구결과가 '이달의 뉴스'로 홍보되어 세계 과학계의 주목을 받고 있다.

□ 편집인 배렛 박사(Dr. Barrett)는 2월호에 게재된 논문을 '이달의 뉴스'로 선정한 사유를 "납에 의한 신장손상의 원인제시 (Seeds of Toxicity? Erythrocytes and Lead-Associated Kidney Damage)"라는 제목 하에 다음과 같이 기술하고 있다.

□ 많은 역학조사에서 중금속 납의 노출은 만성신장질환을 유발함이 잘 알려져 있는데 혈중 납의 농도가 5 μg/dL 이하의 인구집단에서도 신

장기능이 현저히 손상되어 건강에 심각한 위협이 되고 있다. 그러나 납이 신장손상을 유발하는 정확한 기전이 알려지지 않아 납 관리정책 수립에 어려움이 있었다.

□ 서울대학교 연구팀은 혈액내 적혈구와 신장조직의 상호작용을 통하여 신장질환이 유발된다는 가설을 시험관 및 동물실험을 통하여 규명하였다. 기존에는 신장 단일조직에 초점을 맞추어 납의 독성을 연구하였지만, 서울대 연구팀은 시스템 생물학 (Systems Biology) 관점에서 조직간 유기적인 작용을 통하여 신장독성이 유발됨을 증명한데 큰 의의가 있다.

□ '이달의 뉴스'에는 에모리대학 교수의 게재논문에 대한 코멘트도 언급되고 있다. 이 연구에서 증명한 시험관, 동물시험의 결과를 인체 역학조사를 통하여 확인하는 것은 매우 흥미롭고 중요한 과제이다. 환경내 납 오염이 인류 건강을 심각하게 위협함을 고려할 때, 납 노출에 의한 신장질환을 제어할 수 있는 새로운 방안 제시가 가능할 것이다.

□ 권소연씨는 서울대 약대 정진호 교수 지도하에 2013년 서울대 약대 석사 졸업 후, 현재 삼성바이오에피스 (Samsung Bioepis)에 근무하고 있다.

< 첨부 자료 >

1. 연구자 사진 및 인적사항
2. '이달의 뉴스' 기사, pdf 파일
3. 게재논문 표지, pdf 파일

< 연구자 사진 및 인적사항 >

권소연 : 2013년 서울대약대 석사 졸업
현재 소속 : 삼성바이오에피스 근무
연락처 : 사무실) 032-455-6716

< '이달의 뉴스' 기사 >

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Seeds of Toxicity?

Erythrocytes and Lead-Associated Kidney Damage

Environmental lead contamination lingers despite decades of sharply declining use and release of the metal. As a consequence, low-level lead exposure remains common, with an average adult blood lead level in the general U.S. population of 1–2 μg/dL.[1,2] Lead affects numerous organ systems, but specific mechanisms of damage are not always known. The authors of a new study in *EHP* present a hypothesis to explain lead-related toxicity in the kidney and support it with detailed *in vivo* and *in vitro* data.[1]

Current evidence suggests that kidney damage can occur at blood lead levels as low as 5 μg/dL.[2] Specific populations, including people with preexisting kidney disease, diabetes, or hypertension, may be at even greater risk of effects of low-level lead exposure.[2,3] Both *in vivo* and *in vitro* data highlight oxidative stress as a factor in lead-associated kidney damage, but it has been unclear how the stress is generated.[1,2]

A new study explores the role that aging red blood cells may play in lead-related kidney toxicity.
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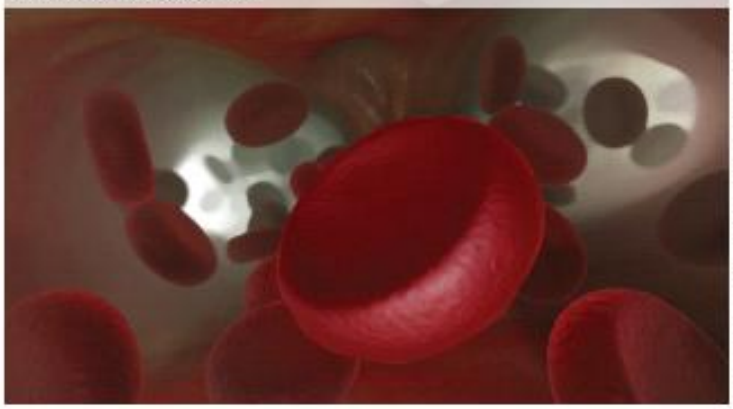

In the current study, the researchers wove observations from previous investigations into a testable hypothesis. They took into account deposition of iron—presumably from iron-rich red blood cells (erythrocytes)—in kidneys of individuals with renal disorders. They also considered the kidney's role in clearing erythrocytes from circulation as the cells become old or damaged.

During a process called erythrophagocytosis, aging erythrocytes are enveloped and broken down by other cells. Erythrophagocytosis occurs primarily in cells of the spleen and liver, but proximal tubular epithelial cells in the kidney also have this capability. The signal that an erythrocyte needs to be removed from circulation comes from a compound called phosphatidylserine (PS). In a normal, healthy erythrocyte, PS is an internal cellular component, with no direct contact with the cell's outer environment. Aged and damaged red blood cells begin to shift PS to the outer surface. Members of this research team previously found that lead exposure was associated with a surge in PS externalization, followed by enhanced erythrophagocytosis in the spleen.[4]

In this study the researchers hypothesized that lead exposure increases the number of PS-tagged erythrocytes, which are then consumed by the proximal tubular epithelial cells in the kidney. They further hypothesized that iron from the erythrocytes accumulates in the kidney cells, where it triggers the formation of reactive oxygen species, the first step in oxidative damage.

The ultimate biological outcome of toxic exposures is frequently a combination of two factors—individual responses of multiple tissues and, subsequently, complex interactions of altered tissues—says study coauthor Jin-Ho Chung, a professor in the College of Pharmacy at Seoul National University. "We considered that lead-associated nephrotoxicity must be interpreted in the context of systems biology, rather than as sole and isolated damage to the kidney," Chung says.

Chung and his colleagues conducted a series of *in vitro* experiments with HK-2 cells[5] and erythrocytes derived from volunteers' blood samples. They showed that, in the absence of erythrocytes, the viability of lead-exposed HK-2 cells was not significantly different from the viability of unexposed HK-2 cells. A separate experiment demonstrated increased PS externalization in lead-exposed erythrocytes. And when lead-exposed erythrocytes were co-cultured with unexposed HK-2 cells, the HK-2 cells not only phagocytized the erythrocytes, but also showed increased production of reactive oxygen species, diminished viability, and greater expression of genes associated with kidney damage.[1]

Next, the *in vitro* findings were tested in rats. Samples of blood, spleen, and kidney tissue were collected from rats exposed to 0 or 1,000 ppm lead in drinking water for 12 weeks, then used for biochemical and histological analyses. These analyses revealed statistically significant changes consistent with kidney damage. A second 12-week experiment included tissue samples from rats that received 0, 250, or 1,000 ppm of lead acetate in drinking water; these results also supported the *in vitro* findings. The *in vivo* results also reflected two types of nephrotoxicity seen in epidemiological studies: proximal tubular nephropathy and interstitial fibrosis.[1]

William McClellan, a professor of medicine at Emory University in Atlanta who was not involved in the study, cautions against immediately extrapolating the findings to human health. He also notes that the findings will need replication. McClellan says, "I have a feeling that if they come up with comparable findings [for confirmation], there will be some strong interest in doing some human studies."

Julia R. Barrett, MS, ELS, a Madison, WI–based science writer and editor, has written for *EHP* since 1996. She is a member of the National Association of Science Writers and the Board of Editors in the Life Sciences.

■ REFERENCES AND NOTES

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5. HK-2 cells are a type of human proximal tubular epithelial cell.

< 게재논문 표지 >

Research

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Erythrophagocytosis of Lead-Exposed Erythrocytes by Renal Tubular Cells: Possible Role in Lead-Induced Nephrotoxicity

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Background: Nephrotoxicity associated with lead poisoning has been frequently reported in epidemiological studies, but the underlying mechanisms have not been fully described.

Objectives: We examined the role of erythrocytes, one of the major lead reservoirs, in lead-associated nephrotoxicity.

Methods and results: Co-incubation of lead-exposed human erythrocytes with HK-2 human renal proximal tubular cells resulted in renal tubular cytotoxicity, suggesting a role of erythrocytes in lead-induced nephrotoxicity. Morphological and flow cytometric analyses revealed that HK-2 cells actively phagocytized lead-exposed erythrocytes, which was associated with phosphatidylserine (PS) externalization on the erythrocyte membrane and generation of PS-bearing microvesicles. Increased oxidative stress and up-regulation of nephrotoxic biomarkers, such as NGAL, were observed in HK-2 cells undergoing erythrophagocytosis. Moreover, TGF-β, a marker of fibrosis, was also significantly up-regulated. We examined the significance of erythrophagocytosis in lead-induced nephrotoxicity in rats exposed to lead via drinking water for 12 weeks. We observed iron deposition and generation of oxidative stress in renal tissues of lead-exposed rats, as well as the histopathological alterations such as tubulointerstitial lesions, fibrosis, and up-regulation of KIM-1, NGAL, and TGF-β.

Conclusions: Our data strongly suggest that erythrophagocytosis and subsequent iron deposition in renal tubular cells could significantly enhance nephrotoxicity following lead exposure, providing insight on lead-associated kidney damages.

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Introduction

Although environmental lead contamination has declined significantly since the 1970s, lead exposure is still observed in children and industrial workers, and even in the general population (Hernberg 2000). The average adult blood lead level (BLL) is 1–2 μg/dL, and the U.S. Centers for Disease Control and Prevention (CDC) defines lead poisoning as a BLL > 10 μg/dL (0.5 μM) (CDC 1997). Epidemiological and toxicological studies have reported lead-induced toxicity in the nervous, cardiovascular, and renal systems [Agency for Toxic Substances and Disease Registry (ATSDR) 2007]. The association between lead exposure and nephrotoxicity has been well-established, even in a population with BLLs as low as 5 μg/dL (Ekong et al. 2006). Damage in kidney function is associated with albuminuria, reduced glomerular filtration rate, and decreased creatinine clearance in lead-exposed populations (Fadrowski et al. 2010; Navas-Acien et al. 2009). Histopathologically, renal impairment associated with lead poisoning is characterized by proximal tubular nephropathy, glomerular sclerosis, and fibrosis in peritubular and interstitial lesions (Cramér et al. 1974; Diamond 2005; Goyer 1989; Loghman-Adham 1997).

Oxidative stress has been suggested to be the most convincing mechanism underlying lead-associated nephrotoxicity (Daggett et al. 1998; Wang et al. 2009). Pro-oxidant and antioxidant balance, along with decreased glutathione and increased lipid peroxidation, occurs in the kidney following lead exposure in animal models (Daggett et al. 1998; Liu et al. 2012b; Patra et al. 2001; Wang et al. 2010). There have been several attempts to determine how lead increases oxidative stress in the kidney (Stucchiotti et al. 2009; Wang et al. 2009, 2011), but the exact mechanism(s) has not been clearly elucidated.

There is increasing evidence (Madsen et al. 1982; Mimura et al. 2008; Sheerin et al. 1999; Trump et al. 1969) that the kidney may play a role in the clearance of erythrocytes. Infiltration of erythrocytes has been observed in proximal tubules and tubular lumen of renal biopsies from patients with acute glomerulonephritis and hematuria (Trump et al. 1969) as well as in those from patients with acute renal failure (Mimura et al. 2008). Iron deposition in the kidney was also found in patients with various renal diseases (Wang et al. 2001), suggesting that the retention of iron-rich erythrocytes in the kidney may play a role in the pathogenesis of kidney diseases. Proximal tubular epithelial cells are capable of phagocytizing and degrading erythrocytes (Madsen et al. 1982; Sheerin et al. 1999), a phenomenon known as erythrophagocytosis. Erythrophagocytosis, which is primarily carried out by macrophages in the spleen and liver (Knutson and Wessling-Resnick 2003; Otogawa et al. 2007), occurs when aged or damaged erythrocytes are phagocytized and cleared from systemic circulation. This process is mediated by externalized phosphatidylserine (PS) on the outer membrane (Kobayashi et al. 2007; Mercer and Helenius 2008) and by PS-bearing microvesicles (MVs) (Knutson and Wessling-Resnick 2003; Otogawa et al. 2007). Proximal tubule cells have been reported to actively phagocytize erythrocytes in renal injury (Madsen et al. 1982; Sheerin et al. 1999), but the toxicological significance of this process in the etiology of heavy metal–associated renal diseases remains to be established.

More than 99% of blood lead accumulates in erythrocytes, suggesting that erythrocytes may be a major target of systemic lead poisoning (Hernández-Avila et al. 1998; Schütz et al. 1996). We recently demonstrated that lead significantly increased PS externalization in erythrocytes and enhanced erythrophagocytosis by macrophages in the spleen (Jang et al. 2011). Fowler et al. (1980) observed iron deposition in renal proximal tubule cells, along with lead-associated histopathological lesions in the kidney, following administration of lead via drinking water to rats. In the present study we examined the role of erythrocytes in lead-induced nephrotoxicity *in vitro* in a co-culture system as well as *in vivo* in rats. On the basis of available evidence, we hypothesized that lead-induced PS externalization in erythrocytes promotes erythrophagocytosis by renal tubular cells, contributing to lead-associated kidney damage.

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