ナノマテリアルの健康影響の評価手法に関する総合研究

平成 20 年度　厚生労働科学研究費補助金

化学物質リスク研究事業

H20 － 化学 － 一般 － 008

平成 20 年度　総括・分担研究報告書

研究代表　　武田　健

平成 21 年 3 月

目 次

厚生労働科学研究費補助金（化学物質リスク研究事業）
平成20年度総括研究報告書

ナノマテリアルの健康影響の評価手法に関する総合研究

研究代表者：武田　健（東京理科大学薬学部教授）

研究要旨：本プロジェクトではナノマテリアルを妊娠期曝露後、母獣、出生した子における体内挙動を長期間にわたって把握するとともに、生体影響評価手法を病理学的に、また、薬理学的、物理化学的、分子生物学的解析手段をもちいて確立する。げっ歯類の他に胎盤特性（構造および薬物透過性）や化学物質応答性がヒトと高い共通性を持つ霊長類への影響を比較検討し、ヒトへの外挿可能な健康影響評価手法を確立する。

研究分担者：中村　伸
（京都大学霊長類研究所　助教）

A. 研究目的

ナノマテリアルはナノテクノロジー基盤素材として活用が期待されている。その物理化学的な特性により、肺や皮膚、腸管から体内に取り込まれ、生体に影響が及ぶことが報告されている。しかし、母親から子への移行と出生後の子への健康影響に関する報告はほとんどない。本研究ではナノマテリアルのヒト健康影響の評価手法確立を目指し、げっ歯類及び霊長類の実験系において次世代を含め健康影響評価手法に関する研究を行う。

B. 研究目的

<u>げっ歯類（マウス・ラット）を用いた研究</u>

ナノマテリル：非意図的生成ナノ粒子としてディーゼル排気微粒子（DEP）、意図的ナノ粒子生成物としてカーボンブラック、酸化チタン、フラーレン、カーボンナノチューブを優先的に使用する。粒径、形状、表面加工の異なるものを用いた。

ナノマテリアルの分散状態解析：様々な溶媒中でナノマテリアルおよび水溶化させたものの分散状態をUV-vis-NIR吸収／発光、ラマンスペクトル、AFM、動的光散乱、ξ-電位測定装置等を用いて解析した。

ナノマテリアルの検出・同定：細胞内、組織内ナノマテリアルの検出・同定は、TEM、STEM、FE-SEM、X線スペクトロ測定装置(EDS)を用いて解析した。

ナノマテリアルの動態解析：妊娠マウス及びラットにナノマテリアルを経気曝露あるいは皮下投与し、主に出生仔の脳、生殖器への移行を上記検出法により検討した。

ナノマテリアルの生体影響解析：妊娠マウス・ラットにナノマテリアルを投与後出生した仔の脳神経系への影響について、行動薬理学的試験、脳内モノアミン類の測定および脳内遺伝子発現変動の解析を行った。雄性生殖系への影響に

ついては、精子産生機能、精子および精巣の超微形態を観察して評価した。

霊長類を用いた評価試験

主にアカゲザルを利用して

ナノマテリアルの胎盤−胎児への移行、出生仔への影響を脳・生殖器・脂肪組織など主要組織・部位での遺伝子発現プロファイル解析、病理解析および細胞内局在解析などを行うための様々な条件を検討し、サンプルを採取した。詳細は別紙参照(中村伸報告)。

(倫理面への配慮)

げっ歯類動物実験は、東京理科大学倫理委員会での承認を得、文部科学省「研究機関等の動物実験等の実施に関する基本指針」、東京理科大学動物実験指針を遵守して行った。ナノ粒子の安全性が不明であることから、P2 プラスレベルの実験に準じた作業手順を実施した。

サルモデルでの実験は京都大学霊長類研究所動物実験倫理委員会での承認を得て実施した。霊長類研究所動物実験指針に基づいたサル飼育・管理および実験利用に努め、投与試験に関しては、環境汚染対応が可能な霊長類研究所バイオハザード飼育・実験室で実施した。

C. 研究成果

H20 年度成果

ナノマテリアルの細胞内取り込み

ディーゼル排気微粒子(DEP)、カーボンブラック、酸化チタンナノ粒子を株化精巣ライディッヒ細胞に曝露し in vitro での影響を評価した。微粒子はいずれも速やかに細胞内に取り込まれたが、細胞毒性および酸化ストレス応答因子の HO-1 遺伝子の誘導作用は粒子により差が見られた。

酸化チタンナノ粒子の胎生期曝露による影響検討

酸化チタンを皮下投与した妊娠マウスからの出生仔について、6 週齢時に精巣組織の TEM 観察、および FE-SEM/EDS による解析を行ったところ、Ti 粒子が検出・同定された。曝露群の精巣組織ではセルトリ細胞の減少やミトコンドリアの損傷が認められた。1 日精子産生数の有意な低下が認められた。脳組織についての同様の解析では、嗅球、大脳皮質、海馬などの部位に酸化チタン粒子が検出・同定された。組織学的検討では、曝露群の組織で嗅球僧帽細胞のカスパーゼ-3(アポトーシスのマーカー)の発現が亢進していることが明らかとなった。

霊長類を用いた検討(中村　伸)

別紙参照

D. 考察

ナノマテリアルの有害性と健康への影響の実態の一部が実験的に明らかになった。ナノマテリアルは脆弱性集団の妊娠期母獣に強く影響を及ぼし、産仔が強く影響を受ける。すなわち次世代への影響が強く認められる。以上の結果は予防法、治療法を考える上で貴重な情報と考えられる。

E. 結論

我々が得た結果は、ナノマテリアルが妊娠中の動物体内に入ると、母から仔に移行し、未発達な脳血液関門、精巣血液関門などを通過し、周辺の細胞に影響を及ぼすこと、生まれてから成長する

過程で様々な症状として現れること、それらは時として重大な疾患の発症、増悪化に繋がる恐れがあることを示唆している。

F. 健康危機情報

健康危険情報について、下記のとおり通報する。

(1)健康危険情報

現在、国際的にナノテクノロジーの基盤材料であるナノマテリアルの毒性の有無とその程度が議論され始めている。我々はカーボンブラック、カーボンナノチューブ、フラーレン、酸化チタンなど工業的に生産される様々なタイプのナノマテリアルの健康への影響、特に次世代を担う子供たちへの影響を中心に研究に取り組んできた。この過程で、酸化チタンを妊娠マウス皮下に投与すると、酸化チタンナノ粒子が産仔の脳に移行し、脳末梢血管周囲に異常が認められ、脳の特定の部位に集中的にアポトーシス像が認められた。酸化チタン粒子は精巣にも取り込まれ、様々な機能変化を引き起こしていた(Takeda, *et al.* JHS)。また、未発表データではあるが、脳内のモノアミン系の代謝異常も認められている。さらに、網羅的遺伝子発現解析並びに選択的遺伝子発現解析の結果からも様々な異常が明らかになってきている。

(2)情報源

研究者名:Ken Takeda, *et al.*

タイトル:Nanoparticles Trasferred from Pregnant Mice to Their Offspring Can Damage the Genital and Cranial Nerve Systems

雑誌名:Journal of Health Science, 55(1) 95-102, 2009

(3)情報に関する評価・コメント

グレード A 情報(事の重大性から、国民に知らせるべき情報の重要性として判断した場合)

グレード B 情報(時間的緊急性ということから判断した場合)

G. 研究発表

1. 論文発表

Takeda, K., Suzuki, K., Ishihara, A., Kubo-Irie, M., Fujimoto, R., Tabata, M., Oshio, S., Nihei, Y., Ihara, T., Sugamata.M. (2009) Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems.
J. Health Sci., 55(1), 95-102.

Xu G., Umezawa M., and Takeda K. (2009) Early Development Origins of Adult Disease Caused by Malnutrition and Environmental Chemical Substances.
J. Health Sci., 55(1), 11-19.

Yokota, S., Mizuo, K., Moriya, N., Oshio, S., Sugawara, I., and Takeda, K. (2009) Effect of prenatal exposure to diesel exhaust on dopaminergic system in mice.
Neurosci Let. 449, 38-41.

Komatsu,T., Tabata, M., Kubo-Irie, M., Shimizu, T., Suzuki, K., Nihei, Y., and Takeda, K. (2008) The effects of nano-particles on mouse testis Leydig cells in vitro.
Toxicol. In Vitro. 22, 1825-1831.

Tokuhiro, K., Hirose, M., Miyagawa, Y., Tsujimura, A., Shinji Irie, A., Isotani, A., Okabe, M., Toyama,Y., Ito, C., Toshimori,K., Takeda, K., Oshio,S., Tainaka,H., Tsuchida,J,, Okuyama, A., Nishimune,Y.,

and Tanaka, H. (2008) Meichroacidin containing the MORN motif is essential for spermatozoa morphogenesis.
J. Biol. Chem. 283(27)19039-19048.

Satou, K., Nonaka, Y., Ohashi, N., Shimizu, M., Oshio, S., and Takeda, K. (2008) The effects of in uter exposure to a migrant, 4,4'-butylidenebis (6-t-butyl –m-cresol), from nitrile-butadiene rubber gloves on monoamine neurotransmitter in rats.
Biol. Pharm. Bull. ;31(12):2211-5.

Ono, N., Oshio, S., Niwata, Y., Yoshida, S., Tsukue, N., Sugawara, I., Takano, T., and Takeda, K. (2008) Detrimental effects of prenatal exposure to filtered diesel exhaust on mousespermatogenesis.
Archives of Toxicology. 82 (11): 851-859.

Umezawa M., Sakata C., Tanaka N., Kudo S., Tabata M., Takeda K., Ihara T., Sugamata M. (2008) Cytokine and chemokine expression in a rat endometriosis is similar to that in human endometriosis. Cytokine 43 (2), 105-109.

Umezawa M., Sakata C., Tabata M., Tanaka N., Kudo S., Takeda K., Ihara T., and Sugamata M. (2008) Diesel exhaust exposure enhances the persistence of endometriosis model in rats.J. Health Sci., 54(4),503-507.

K. Suzuki, T. Komatsu, M. Kubo-Irie, M. Tabata, K. Takeda, Y. Nihei.(2008) Interaction between Diesel Exhaust Particles and Cellular Oxidative Stress. Applied Surface Science, 255, 1139-1142.

N.Fukuhara, K. Suzuki, K. Takeda, Y. Nihei. (2008) Characterization of Environmental Nanoparticles.
Applied Surface Science, 255, 1538-1540.

武田健、菅又昌雄「未知なる遭遇—ナノマテリアルの健康影響～次世代影響を中心に」:ファルマシア 45(3)245-250 (2009)

2. 学会発表

梅澤雅和、田中奈央美、久藤清香、坂田千佳、田畑真佐子、武田健、井原智美、菅又昌雄:実験的ラット子宮内膜症モデルに及ぼすディーゼル排ガス曝露の影響, 自然免疫の最前線・3学会合同大会(第73回日本インターフェロン・サイトカイン学会、第19回生体防御学会、第 45 回補体シンポジウム(2008年) 札幌(北海道)

梅澤雅和、田畑真佐子、武田健、井原智美、菅又昌雄:ディーゼル排ガス曝露は実験的ラット子宮内膜症モデル病変を持続させる, 第49回大気環境学会 (2008年) 金沢(石川県)

M. Sugamata , T. Ihara, K. Takeda , MATERNAL EXPOSURE TO TITANIUM DIOXIDE NANO-PARTICLES DAMAGES NEWBORN MURINE BRAINS , 21th European Congress of Perinatal Medicine September 10-13, 2008 Istanbul, Turkey

田中奈央美、梅澤雅和、久藤清香、坂田千佳、田畑真佐子、武田健、井原智美、菅又昌雄:ラット子宮内膜症モデルに及ぼすディーゼル排ガス曝露の影響, 第52回日本薬学会関東支部会, 2008年10月, (千葉)

小野なお香、押尾茂、菅原勇、高野裕久、武田健:ディーゼル排ガス胎仔期曝露はマウス精子形成過程を障害する, 第52回日本薬学会関東支部会, 2008年10月, (千葉)

高橋光、田井中均、武田健、押尾茂: 精巣特異的マイクロアレイと精巣毒性評価, 第52回日本薬学会関東支部大会, 2008年10月, (千葉)

高橋雄大、水尾圭祐、岡田悠佳、篠塚崇徳、武田健: 酸化チタンナノ粒子胎仔期曝露が脳内モノアミン系に及ぼす影響, 日本薬学会第129年会, 2009年3月(京都)

高橋光、田井中均、田中宏光、西宗義武、武田健、押尾茂: 精巣特異的遺伝子発現解析に基づく精巣毒性評価, 日本薬学会第129年会, 2009年3月(京都)

梅澤雅和、武田健、井原智美、菅又昌雄:ディーゼル排気微粒子の胎仔期曝露はラットの肺におけるアレルギーを亢進させる, 日本薬学会第129年会, 2009年3月(京都)

田中奈央美、梅澤雅和、田井中均、武田健、井原智美、菅又昌雄:マイクロアレイを用いた子宮内膜症の発生初期に関与する遺伝子発現の解析, 日本薬学会第 129 年会, 2009年3月(京都)

H. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金 （化学物質リスク研究事業）
平成 20 年度分担研究報告書

霊長類に対するナノマテリアルの影響評価

分担研究者(グループ長):中村　伸(京都大学霊長類研究所、助教)

研究要旨:本研究ではサルモデルを駆使して、ヒトに外挿可能なナノマテリアルの生体影響とその評価手法に関する研究を展開する。具体的には、化学物質脆弱集団であるサル胎仔・乳幼仔について、非意図的および意図的に産生されているナノ物質がもたらす生体影響を明らかにすると共に、その分子基盤を明らかにする。同時に、サルモデルを利用したナノ物質リスク評価系について、RNA ゲノミクスを主体にしたアセスメント系の確立も図る。

分担研究者：光永総子（京都大学霊長類研究所、教務補）
リサーチ・レジデント：上岩美幸（化学物質リスク研究推進事業、リサーチ・レジデント）

研究目的

本研究ではサル類の生物・生理学的特性がヒトに酷似する点に着目し、サルモデルを駆使したナノマテリアルのリスク評価研究を展開する。具体的には、化学物質脆弱集団である胎仔および乳幼仔について、組織病理学的、分子細胞生物学的ならびに RNA ゲノミクスの手法で、ナノマテリアルが引き起こす生体影響とその分子基盤を検討する。その目的に沿って、本年度は以下の研究を進めた。

研究方法

サルモデル:今年度は生体影響評価系としてアカゲザルの胎仔モデルを用いることとし、交配で 10 頭の妊娠ザルを得た。次いで、胎仔影響の性差を考慮して、本研究で確立した非侵襲的胎仔性別診断法を活用して(後述)、妊娠ザルをオスおよびメス胎仔用に 2 分した。また、下記のナノマテリアル投与実験には、麻酔を含めた実験ストレスで流産・死産リスクを生じない様に、期間中特段の注意を払ってサルの飼育管理につとめた。

また、乳幼仔モデルについてはオス 4 頭およびメス 4 頭の乳幼仔を、生後 6-8 ヶ月で母親から分離し、ストレス緩和を図るために 2 頭でのペアー飼育など、well-being に留意しながら馴化して実験に備えた。

いずれのサル飼育管理およびサル実験についても、霊長類研究所サル委員会での承認を得ると共に、実施にあたっては、ストレス緩和に努めた。

ナノマテリアル:非意図的ナノ物質としてディーゼル排気ナノ粒子(DEP)を、一方、意図的ナノ物質としてカーボンブラック(CB)を選択した。Tween-60/PBS で DEP および CB の懸濁液を調整後、超音波処理で均一化し、以下の投与に用いた。

投与条件：ナノマテリアルの経皮暴露（化粧品利用）等を想定して、DEP およびCBを、サルの手が届かない背中部の皮内に投与した。妊娠ザルへの投与量は、いずれも18mg/headで、胎盤透過性が高まっている妊娠後期に7-10 日間隔で5-6回投与した。この皮内投与については、我々の以前の研究で、リポソーム性ナノ粒子が投与部位の皮内から徐放的に皮下拡散し、毛細血管に移行して、血流循環する事。さらに、皮内投与したリポソーム性ナノ粒子が、妊娠下では胎盤を経て胎仔に至る事も確認している（文献1、2）。

生体影響の評価：胎仔期暴露サル試料を得るために、上記条件で妊娠アカゲザル母体に DEP や CB を投与し、最終投与後、自然分娩で得た新生仔を胎仔期ナノマテリアル暴露サルとして、血液、脳（小脳、海馬、大脳皮質、嗅球など）、生殖器、その他主要組織を採取した。組織化学的観察には固定組織試料を、分子細胞生物学的検討には生組織断片の液体窒素凍結試料を用意した。RNA Genomics 解析用には生組織小断片をRNALatter 処理した試料を調え、特殊な条件でDNAフリーな高純度RNAを調製した。次いで、種々のサル機能遺伝子発現の変動を定量的に解析するReal-time RT-PCR の条件化を図った。さらに、遺伝子発現の変動を網羅的に解析するために、種々DNA アレイ/チップを試し、生体影響評価系としてのトキシコゲノミクス解析を試みた。この二つの RNA/遺伝子発現レベルの解析手法を統合した、RNA Genomics による生体影響評価系を検討した。

研究成果および考察

サル胎仔の雌雄判別：母体血流に存在する微量の胎仔 DNA に着目して、母体血液中のY（雄性）染色体特異的 gene の SRY-DNA の高感度検出法を、nested Real-time PCR で確立した。

この方法で妊娠初期の 42 日から、母体血液 2ml 程度で胎仔の雌雄判定が可能になった（リサーチ・レジデント上岩美幸との共同研究、研究発表-1）。従来は超音波を利用した胎仔性別判定が実施されていたが、サル胎仔のオス生殖器が小さいため、高性能の超音波装置を駆使しても妊娠後期でないと性別判定が困難であった。一方、本研究で確立した SRY nested Real-time PCR 法は、超音波装置など特別な設備なしに、妊娠早期においてサル胎仔の雄雌判定を可能にした点で画期的といえる。ことに、本研究においては、霊長類胎仔におけるナノマテリアルのリスク発現の雄雌差について、質的・量的比較解析が非常に容易になり、本研究の加速的推進の要素となった。

サルモデル試料：上記の妊娠アカゲザルを用い、母親に DEP や CB を投与し、胎仔期にナノマテリアル暴露された組織・試料を、雌雄それぞれ 2 頭分得た。同時に、ナノマテリアル非暴露のコントロール組織・試料も雌雄それぞれ 2 頭分用意した。

また、アカゲザルの雌雄乳幼仔各 2 頭に、DEP や CB の相当量を 7-10 日間隔で5-6回投与し、安楽死後、組織化学的観察、分子細胞生物学的検討および Real-time RT-PCR およびトキシコゲノミクス解析用の試料を調えた。非投与の雌雄乳幼仔についても数頭からコントロール試料を採取した。

さらに、アカゲザル成獣の雌雄につい

てもDEPやCBを投与し、非投与のコントロール個体とともに安楽死後、必要試料を調製した。

これらの試料を、胎仔期、乳幼仔期および成獣期におけるナノマテリアル暴露の生体影響の比較解析および評価系検討等、次年度以降の研究にも用いる。

生体影響の評価系（主にRNA Genomics）：Real-time RT-PCRは機能遺伝子の定量的発現解析に不可欠であるため、SYBR Greenを用いたPCR産物の高感度検出条件を確立した。同時に、種々のサル用PCR Primerを設計して、100種以上の機能遺伝子について、Real-time RT-PCRでの定量的発現解析を可能にした。

さらに、1万以上の遺伝子の発現を一括して網羅的に検討するための、サル用DNAアレイ/チップについて検討した。絞り込んだ候補の3者（CodeLink, Filgen, 3D gene）について種々の観点から調べ、本評価系に適したDNAアレイ/チップとして東レの3D geneを特定した。

現在、これらの遺伝子発現解析手法を駆使して、DEPやCBの胎仔影響について検討している。これまで、脳（海馬）、褐色脂肪組織、リンパ節、生殖組織などで興味深い遺伝子発現イベントが見られている。同時に、Real-time RT-PCRとDNAアレイ/チップを組み合わせた統合的RNA Genomicsを検討し、定量性と網羅性を兼ね備えた新たなナノマテリアル生体影響評価系の開発を目指している。

研究発表

1. Ueiwa M, Mitsunaga F, Nagatono T, & Nakamura S. : Macaque Fetus Sex Determination by Nested Real-time PCR, Exp. Anim.(Submitted) .

2. Yamate J, Izawa T, Kuwamura M, Mitsunaga F, & Nakamura S.: Vasoformative Disorder, Resembling Littoral Cell Angioma of the Spleen in a Geriatric Japanese Macaque (Macaca f uscate). Vet Pathol. (in press).

3. Jeong A.-R, Nakamura S & Mitsunaga F: Gene expression profile of Th1 and Th2 cytokines and their receptors in human and nonhuman primates J. Med. Primatol.,37:290-296 (2008).

4. 光永総子、中村　伸：サルモデルを用いた機能性食品の評価試験、FFI J. Jpn. , 213:719-726 (2008).

文献

1. Hirano M, Nakamura S, Mitsunaga F, Shimizu K, Imamura T, Placental Transfer of Maternally Administered Liposome-DNA Complex into Fetuses in a Pregnant Primate Model, J. Gene Med, 4: 560-566 (2002).

2.光永総子、中村　伸、平野　真、清水慶子、今村隆寿：霊長類胎盤構造の特徴：遺伝子治療剤の胎盤通過の視点から：、霊長類研究、17:35-45(2001)

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書 籍 名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeda K., Suzuki K., Ishihara A., Kubo-Irie M., Fujimoto R., Tabata M., Oshio S., Nihei Y., Ihara T., Sugamata M.	Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems.	J. Health Sci.	55(1)	95-102	2009
Xu G., Umezawa M., and Takeda K.	Early Development Origins of Adult Disease Caused by Malnutrition and Environmental Chemical Substances.	J. Health Sci.	55(1)	11-19	2009
Komatsu,T., Tabata, M., Kubo-Irie, M., Shimizu, T., Suzuki, K., Nihei, Y., and Takeda, K.	The effects of nanoparticles on mouse testis Leydig cells in vitro.	Toxicol. In Vitro.	22	1825-1831	2008
光永総子, 中村　伸	サルモデルを用いた機能性食品の評価試験	FFI J. Jpn.	213	719-726	2008
Jeong A.-R, Nakamura S & Mitsunaga F.	Gene expression profile of Th1 and Th2 cytokines and their receptors in human and nonhuman primates	J. Med. Primatol.	37	290-296	2008

Nanoparticles Transferred from Pregnant Mice to Their Offspring Can Damage the Genital and Cranial Nerve Systems

Ken Takeda,*,[a] Ken-ichiro Suzuki,[b] Aki Ishihara,[a] Miyoko Kubo-Irie,[a] Rie Fujimoto,[a] Masako Tabata,[a] Shigeru Oshio,[c] Yoshimasa Nihei,[b] Tomomi Ihara,[d] and Masao Sugamata[d]

[a]Department of Hygiene Chemistry, Faculty of Pharmaceutical Science, [b]Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278–8510, Japan, [c]Department of Hygiene Chemistry, Ohu University School of Pharmaceutical Sciences, 31–1 Misumidou, Tomita-machi, Kooriyama-shi, Fukushima 963–8611, Japan and [d]Department of Pathology, Tochigi Institute of Clinical Pathology, 2308–3 Minamiakatsuka, Nogi, Shimotsuga-gun, Tochigi 329–0112, Japan

(Received September 26, 2008; Accepted October 27, 2008; Published online November 10, 2008)

Nanomaterials are being used increasingly for commercial purposes, yet little is known about the potential health hazards such materials may pose to consumers and workers. Here we show that nano-sized titanium dioxide (TiO_2), which is used widely as a photo-catalyst and in consumer products, administered subcutaneously to pregnant mice is transferred to the offspring and affects the genital and cranial nerve systems of the male offspring. Nanoparticles identified as TiO_2 by energy-dispersive X-ray spectroscopy were found in testis and brain of exposed 6-week-old male mice. In the offspring of TiO_2-injected mice, various functional and pathologic disorders, such as reduced daily sperm production and numerous caspase-3 (a biomarker of apoptosis) positive cells in the olfactory bulb of the brain, were observed. Our findings suggest the need for great caution to handle the nanomaterials for workers and consumers.

Key words —— nanoparticle, titanium dioxide (TiO_2), brain, testis, pregnant mouse, olfactory bulb

INTRODUCTION

Nano-sized particles also known as ultrafine particles, are very tiny particles less than 100 nm in diameter. They are produced daily by activities such as driving, cooking, and generating energy in power plants. Engineered nanomateials are used in sporting goods, tires, stain-resistant clothing, sunscreens, cosmetics, and electonics and will likely be used increasingly in medicine for purposes of diagnosis and drug delivery.[1–4] Nanotoxicology, the evaluation of the safety of engineered nanostructures and nanodevices, is a novel field of toxicology. Materials that are generally thought to be inert may act differently when introduced to the body as nanomaterials.[4–8]

Nanocrystalline titanium dioxide (TiO_2), a non-combustible, odorless powder, is an important material used in commerce. Anatase TiO_2 is currently used in products as diverse as sunscreens and coatings for self-cleaning windows.[9] TiO_2 can generate reactive oxygen species quite efficiently, particularly when exposed to ultraviolet light. The photocatalytic activity of the anatase form of TiO_2 was reported to be higher than that of the rutile form.[10] Gurr and colleagues[11] reported that nano-sized anatase TiO_2 particles induced oxidative DNA damage, lipid peroxidation and micronuclei formations and increased hydrogen peroxide and nitric oxide production in BEAS-2B cells, a human bronchial epithelial cell line, even in the absence of photoactivation. However, the potential toxicity of TiO_2 in the next generation has yet to be examined. In the present study we examined the effects of prenatal exposure to anatase TiO_2 on the genital and cranial nerve systems of male offspring mice.

*To whom correspondence should be addressed: Department of Hygiene Chemistry, Faculty of Pharmaceutical Science, Tokyo University of Science, 2641 Yamazaki, Noda-shi, Chiba 278–8510, Japan. Tel.: +81-4-7121-3618; Fax: +81-4-7121-3784; E-mail:takedak@rs.noda.tus.ac.jp

MATERIALS AND METHODS

Materials —— TiO_2 particles (anatase form, particle size 25–70 nm, surface area 20–25 m^2/g, a purity 99.9 %) was purchased from Sigma-Aldrich (St Louis, U.S.A.).

Animals —— Pregnant Slc : ICR mice (purchased from Japan SLC Inc., Shizuoka, Japan) (6 mice/group) received subcutaneous injections of 100 μl of 1 mg/ml TiO_2 particles in saline plus 0.05 % Tween 80 at 3, 7, 10, and 14 days postcoitum. Control mice were treated on the same schedule with 0.05 % Tween 80. Male offspring were weighed and killed under anesthesia at 4 days or 6 weeks of age. All experimental animals were handled in accordance with institutional and national guidelines for the care and use of laboratory animals.

Organ Weights —— The weights of the testis, epididymis, and seminal vesicle (including prostate, seminal vesicle, and coagulating gland) bilaterally and brain were measured for each animal, and relative weights (weight of the organ/body weight) were calculated in 6-week-old offspring.

Daily Sperm Production (DSP) and Morphological Observation of Testis —— Testicular tissue was thawed and weighed after removal of any extracapsular material from the testis. Testes were homogenized in buffer containing 0.05 % Triton X-100 (Nacalai Tesque, Kyoto, Japan) and 0.2 % Eosin Y (Merck, Darmstadt, Germany). The number of sperm nuclei in each suspension was determined by hemocytometer.

Statistical Analysis —— Data were analyzed by Mann-Whitney U test, and differences were considered significant at $p < 0.05$.

Analysis by Field Emission-type Scanning Electron Microscopy (FE-SEM)/Energy-Dispersive X-ray Spectroscopy (EDS) —— The testis or brain tissue was embedded in epoxy resin for FE-SEM/EDS observation. These samples were cut with thickness of approximately 80 nm with an Ultra-Microtome (Leica EM UC6rt, Leica Microsystems Japan, Tokyo, Japan). Each ultra-thin section was placed on a transmission electron microscopy (TEM) grid (Cu 150-B, Okenshoji, Tokyo, Japan) and analyzed by FE-SEM/EDS (Hitachi High-technology, Tokyo, Japan).

Methods of Immunohistochemical Staining of Caspase-3 —— Tissue samples of olfactory from the TiO_2 treated group and the control group were fixed with 10 % buffered formalin and, after routine dehydration, embedded in paraffin. To detect apoptosis in these olfactory under a light microscope, the immunohistochemical staining for caspase-3 (a common enzymatic biomarker of apoptosis) was performed. Paraffin sections 5-μm thick of olfactory samples were stained immunohistochemically by the streptoavidin-biotin method (Histofine SAB-PO kit, Nichirei, Tokyo, Japan). The primary antibody used was anti-human/mouse caspase-3 (active) rabbit IgG (R&D Systems, Inc., Minneapolis, MN, U.S.A.).

RESULTS

TiO_2 powder size was confirmed by FE-SEM (Fig. 1). Male offspring were killed under anesthesia at 4 days or 6 weeks of age. In order to determine the genital toxicity of TiO_2 particles, body

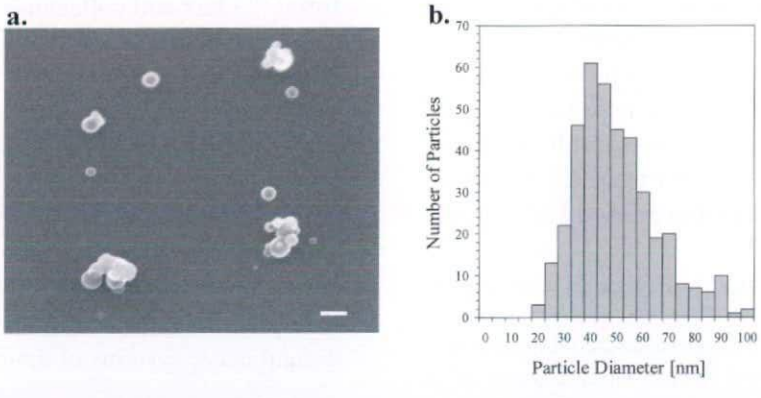

Fig. 1. Distribution of TiO_2 Particle Diameter by FE-SEM

(a) FE-SEM Image of TiO_2 particles (15.0 kV × 80000, Scale bar, 100 nm). (b) Distribution of TiO_2 particle diameters according to FE-SEM analysis. Columns show the diameter of single particles. Diameter of particles was measured on randomly selected area of FE-SEM image.

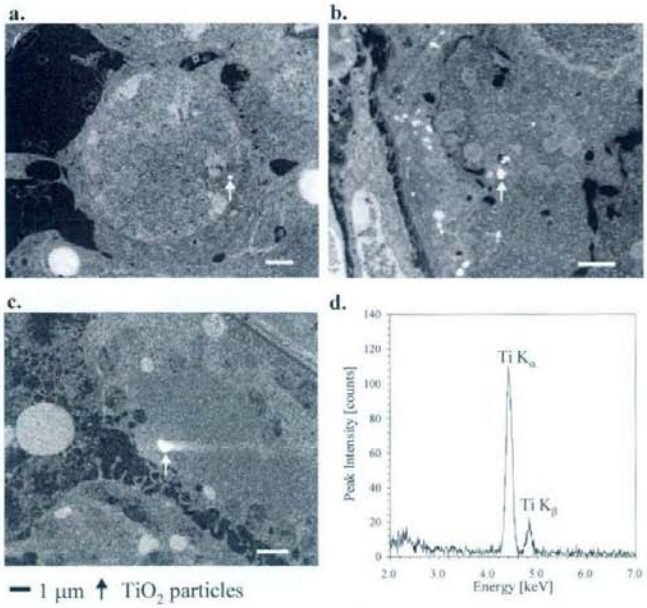

Fig. 2. Detection of TiO_2 Nanoparticles in the Testis of Offspring by EDS

Testes were dissected from 6-week-old mice and fixed. Particles were detected in the cells of testis by TEM and field FE-SEM. The particles were identified as TiO_2 by EDS at 7 kV accelerating voltage, 1×10^{-10} A beam current and 100 sec measurement time. Aggregated TiO_2 nanoparticles (100–200 nm) were detected in spermatids (a), Sertoli cells (b) and Leydig cells (c). Scale bars, 1 μm. TiO_2 particles are indicated by arrows. Particles in the testis were identified respectively as TiO_2 by EDS (d).

and reproduction weights were measured. TiO_2-exposed group had significantly lower body weight (88 % relative to control) and significantly higher weight of epidermis per body weight (117 % relative to control). However, there were no significant changes in the weight of other reproductive organs.

The presence of TiO_2 particles was assessed in testis and brain from 4-day-old and 6-week-old offspring by TEM and FE-SEM. Particles in the testis and brain were identified as TiO_2 by EDS at 7 kV accelerating voltage, 1×10^{-10} A beam current, and 100 sec measurement time.

As shown in Fig. 2, aggregates of TiO_2 nanoparticles (100–200 nm) were detected in Leydig cells, Sertoli cells, and spermatids in the testis at both 4 days and 6 weeks of age. Sperm samples were collected from the cauda epididymis, and sperm motility and morphology were evaluated under phase contrast microscopy. Testes of 6-week-old mice were homogenized, and DSP was examined. Testes were also fixed and stained with standard procedures for examination by light and electron microscopy.

Among 6-week-old mice, the seminiferous tubules of hematoxylin and eosin-stained sections from control mice showed the normal spermatogenic cycle with germ cells and Sertoli cells. Sertoli cells were located regularly in the periphery of the seminiferous tubules and had large nuclei with large nucleoli. Testicular morphology in TiO_2-exposed mice was abnormal compared to that in control mice. In exposed mice, some seminiferous tubules appeared disorganized and disrupted. There were fewer mature sperm in the tubule lumen. The damaged tubules were scattered randomly throughout the testis (Fig. 3). These effects were dependent on the dose of TiO_2 and were significantly higher in the TiO_2 exposed mice than in control mice. DSP per gram of testis, epididymal sperm motility, and the number of Sertoli cells were significantly lower in mice exposed to TiO_2 than in control mice. Sperm morphology did not differ significantly (Fig. 4). These data suggest that prenatal exposure to nano-sized TiO_2 has detrimental effects on mouse spermatogenesis in offspring.

The olfactory bulb and the cerebral cortex (frontal and temporal lobes) of 6-week-old mice were examined by TEM and FE-SEM/EDS. Nano-sized TiO_2 particles were detected in cells in brains of 6-week-old mice exposed prenatally to TiO_2

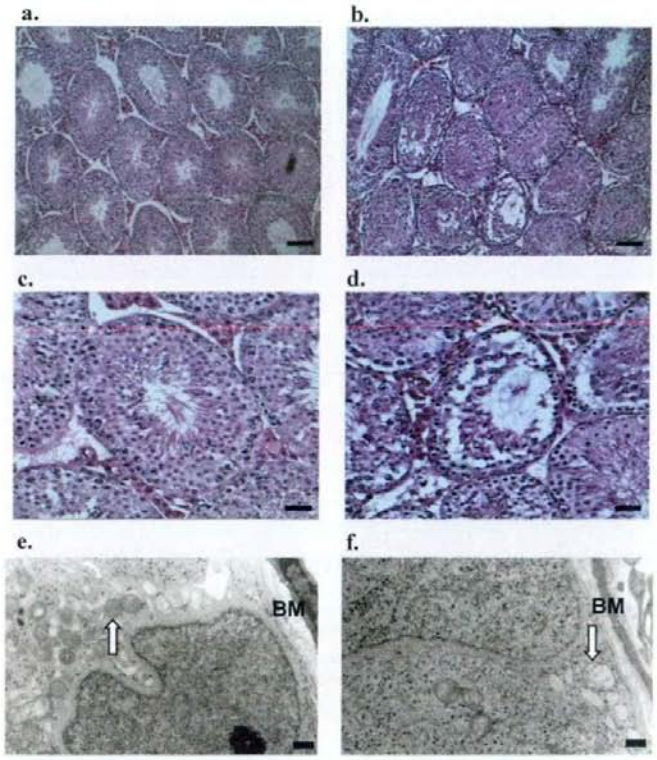

Fig. 3. Morphology of Seminiferous Tubules and Testicular Functions in 6-week-old Mice Exposed Prenatally to TiO_2

Hematoxylin and eosin-stained sections of seminiferous tubules from control mice (a, c) show a normal spermatogenic cycle with germ cells and Sertoli cells. Testicular morphology in TiO_2-exposed mice (b, d) was abnormal compared to that in control mice. Some seminiferous tubules appear disorganized and disrupted. There were fewer mature sperm in the tubule lumen. Damaged tubules were scattered randomly throughout the testis. Scale bars, 100 μm (a, b) and 25 μm (c, d). TEM demonstrating mitochondria (white arrow) of Sertoli cells from control mice (e) and TiO_2-exposed mice (f). Enlargement of mitochondria and disappearance of cristeae were observed (f). Scale bars, 1 μm (e, f). BM; basement membrane.

(Fig. 5, a–e). We believe that the nanoparticles were transferred from the mother to the fetus and moved into the brain because blood-brain barrier was undeveloped.

Numerous cells positive for caspase-3, a common enzymatic marker of apoptosis, were observed under light microscopy in the olfactory bulb of 6-week-old mice exposed prenatally to TiO_2, and the number of caspase-3-positive mitral cells was significantly higher in exposed mice than in control mice (no positive cells, Fig. 6. a, b).

Electron microscopic observations of olfactory bulb revealed that a subset of cells contained cresent-shaped spaces (CSS), which are specific features of apoptosis.[12] Apoptotic granular perithelial (GP) cells, which are scavenger cells that surround vessels in the brain, contained unidentified particulate matter. Occlusion of small vessels and perivascular edema were observed in the prenatally TiO_2-exposed mice.

The abnormalities varied in severity were dependent on the TiO_2 concentration, and were not observed in the control group. These data indicate that prenatal exposure of mice to TiO_2 has a severe negative effect on fetal brain development and carries a risk of various nervous system disorders.

DISCUSSION

We show here that anatase TiO_2 nanoparticles administered subcutaneously to pregnant mice are transferred to and affect the genital and cranial nerve systems of the offspring. These findings suggest that anatase TiO_2 can harm the developing fetus in mice. As we observed in TiO_2-exposed mice, we

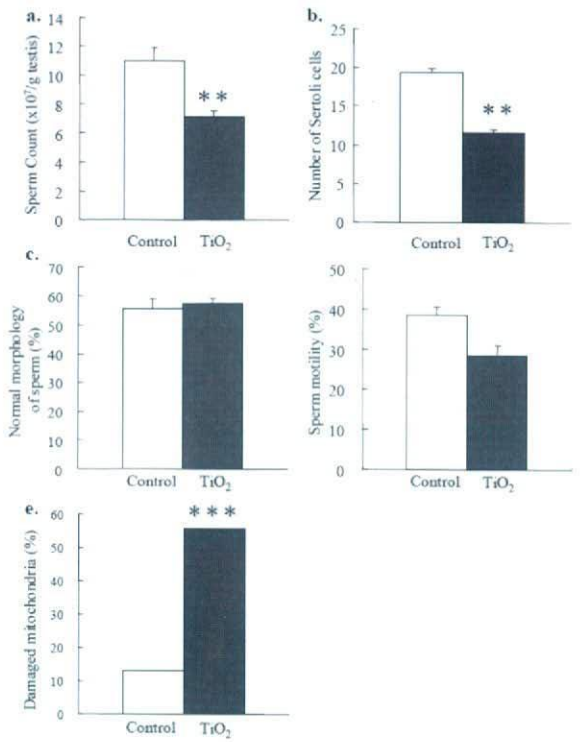

Fig. 4. Effect of Prenatal Exposure to TiO_2 on Seminiferous Tubles and Testicular Functions in 6-week-old Mice

Testis of 6-week-old mice was homogenized on ice, and DSP was determined (a). Sertoli cells in seminiferous tubules were counted (b). Sperm samples were collected from the cauda epididymis, and morphology (c) and sperm motility (d) were determined under phase contrast microscopy. Sertoli cells with damaged mitochondria were counted by TEM (e). Control: $n = 8$, TiO_2: $n = 8$. Presented are the mean ± S.E., where *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

have observed various histologic and functional effects on the male reproductive and central nervous systems in mice exposed prenatally to diesel exhaust (DE)[13–18] and diesel exhaust particles (DEP). The changes in the reproductive and central nervous systems in DE-exposed mice could be reduced by eliminating particles including nano-sized particles with a high-quality filter (unpublished data). Sugamata *et al.*[17] also found that granular perithelial cells, which are scavenger cells, showed signs of apoptosis in the cerebrum and hippocampus of newborn mice exposed prenatally to DE. Furthermore, the cytoplasmic granules of these cells contained nano-sized particles. These observations suggest that exposure of pregnant mice to tiny particles can damage the fetus.

To prevent exposure of the fetus to harmful substances, there is a blood-placenta barrier between the mother and fetus. There is also a blood-brain barrier and blood-testis barrier in the important regions of the brain and genitals, respectively, in adult mice. Our present electron microscopy data indicate that nanoparticles can transfer from pregnant mice into brain and testis of their offspring. These blood barriers are undeveloped or under developed in the fetus, therefore, harmful nanoparticles could easily pass into the brain during the early stages of fetal development.

Nano-sized particles can enter the human body via the lungs and intestines. Whether such particles can penetrate the skin is less clear,[6,7] Kreilgaard[19] suggested that very small TiO_2 particles (*e.g.* 5–20 nm) can penetrate the skin and interact with the immune system. Tinkle *et al.*[20] showed that 0.5- and 1.0-μm particles, in conjunction with motion, penetrate the stratum corneum of human skin and reach the epidermis and, occasionally, the dermis.

There are reports that inhaled or injected nanoparticles enter the systemic circulation[21–23] and migrate to various organs and tissues.[24] If particles enter the body, their distribution is a function of their size and surface characteristics. There may be a critical size beyond which movement of the nanoparticles within the body is restricted. The brain is especially vulnerable to oxygen stress damage, and recent studies have supported our present and previous findings that nanosized particles can be uptaken in brain[25] and enter the central nervous system.[26] Oberdörster *et al.*[27] reported that inhaled nanoparticles could be translocated into brain via the olfactory nerves. Sugamata *et al.*[18] reported previously that specific features of apoptosis were present in Purkinje cells of cerebellum in mice exposed prenatally to DE. In the present study, we observed few apoptotic features in Purkinje cells of TiO_2-exposed mice. DEP and TiO_2 particles may differ in their abilities to induce apoptosis in cerebellum.

Regardless of the particle size, TiO_2 has only minimal effects in adult rodents.[28] However, numerous *in vitro* studies revealed that TiO_2 nanoparticles cause oxidative stress-mediated toxicity in diverse cell types including skin fibroblasts,[29] alveolar macrophages.[30] Long *et al.*[31] showed that mouse microglia engulfed the TiO_2 particles and, for 2 hr, released bursts of reactive oxygen molecules that interfered with mitochondrial energy production. This did not damage the microglia, however, prolonged exposure to such compounds can damage neurons. Greater surface area per mass renders nano-size particles more active biologically than larger particles of the same chemical makeup.

Numerous studies regarding the effects of ul-

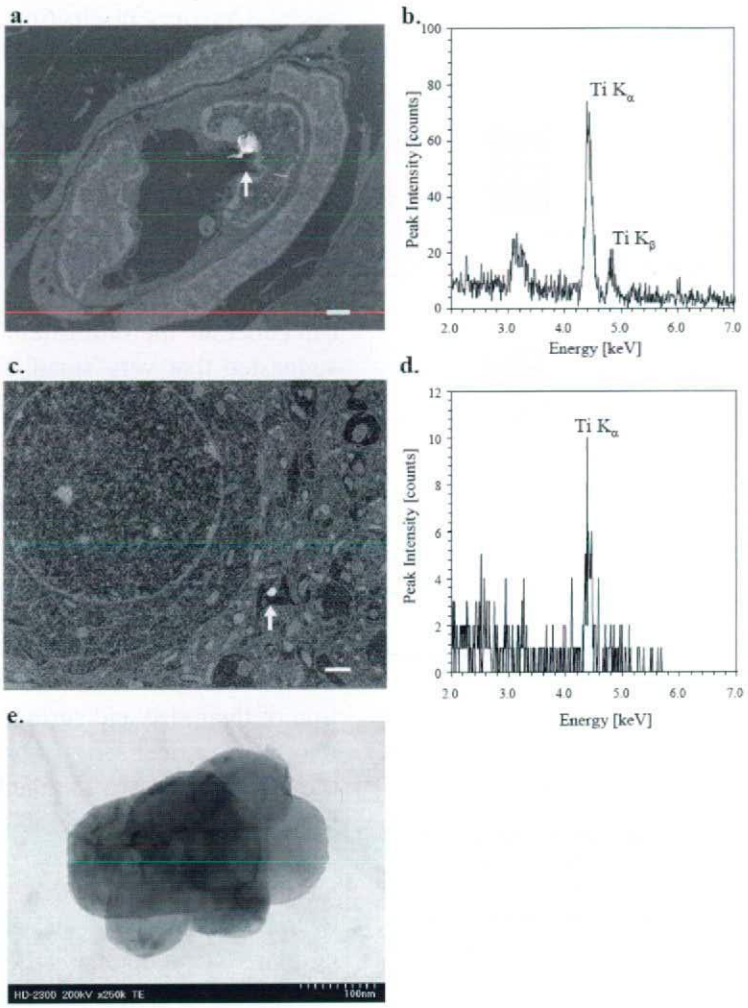

Fig. 5. Detection of TiO_2 Nanoparticles in the Olfactory Bulb and Cerebral Cortex of Brain of Offspring of TiO_2-exposed Mice by EDS

Olfactory bulb and cerebral cortex were dissected from 6-week-old mice and fixed. Particles were detected by TEM and FE-SEM. Photographs demonstrating aggregated TiO_2 nanoparticles (100–200 nm) in endotherial cells of olfactory bulb (a), and nerve cell fibers in cerebral cortex (c). Scale bars, 1 μm. TiO_2 particles are indicated by arrows. Particles in the brain were identified respectively as TiO_2 by EDS at 15 kV (b) and 7 kV (d) accelerating voltage, 1×10^{-10} A beam current and 100 sec measurement time. Electron micrograph demonstrating magnified aggregated TiO_2 particles in nerve cells in cerebral cortex (e).

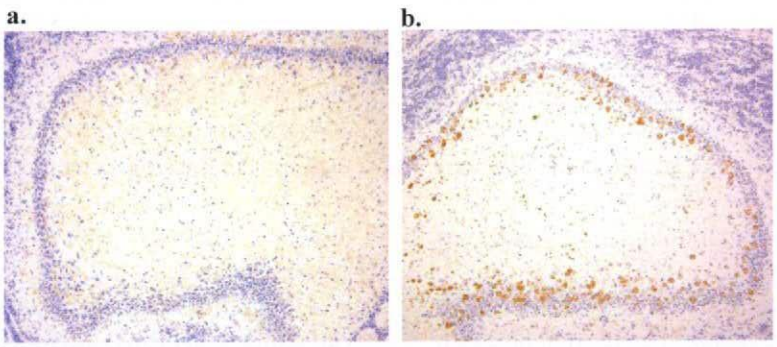

Fig. 6. Immunohistochemical Staining of Caspase-3 in Olfactory Bulb of 6-week-old Mice

(a) Control mice, (b) mice exposed prenatally to TiO_2. Numerous caspase-3 positive mitral cells are visible and the number of positive cells in TiO_2-exposed mice is significantly higher compared with that in control mice.

trafine particle pollutants on respiratory and circulatory systems have been reported. However, little is known about the effect on the genital and central nervous systems. Our present and former findings suggest that widespread use of TiO_2 and other nanoparticles including ultrafine particulates in air might affect unborn children, especially development of their reproductive and nervous systems. Therefore, research into the risk of exposure to nanoparticles, into removal of nanoparticles from the environment, and into methods to protect against toxicity of such particles is important.

Acknowledgements We thank Dr. I. Sugawara of Research Institute of Tuberculosis for help with preparation of testis samples for histology. We thank the graduate and undergraduate students in the Takeda and Nihei laboratories. This work was supported in part by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, a Grant-in Aid for the Private University Science Research Upgrade Promotion Business Academic Frontier Project and a Grant-in Aid for Health and Labour Sciences Research Grants, Research on Risk of Chemical Substances, from the Ministry of Health, Labour and Welfare.

REFERENCES

1) Mazzola, L. (2003) Commercializing nanotechnology. *Nature Biotechnol.*, **21**, 1137–1143.
2) Paull, R., Wolfe, J., Hebert, P. and Sinkula, M. (2003) Investing in nanotechnology. *Nature Biotechnol.*, **21**, 1144–1147.
3) Salata, O. V. (2004) Applications of nanoparticles in biology and medicine. *J. Nanobiotechnology*, **2**, 3.
4) Nel, A., Xia, T., Mädler, L. and Li, N. (2006) Toxic potential of materials at the nanolevel. *Science*, **311**, 622–627.
5) Oberdörster, G., Oberdörster, E. and Oberdörster, J. (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Perspect.*, **113**, 823–839.
6) Donaldson, K., Stone, V., Tran, C. L., Kreyling, W. and Borm, P. J. A. (2004) Nanotoxicology. *Occup. Environ. Med.*, **61**, 727–728.
7) Hoet, P. H. M., Brüske-Hohlfeld, I. and Salata, O. V. (2004) Nanoparticles—known and unknown health risks. *J. Nanobiotechnology*, **2**, 12.
8) Amurao, C. V. (2006) Nanotechnology—It's a small (and scary) world after all. *Occupational Health Tracker*, **9**, 3–6.
9) Wolf, R., Matz, H., Orion, E. and Lipozencic, J. (2003) Sunscreens—the ultimate cosmetic. *Acta Dermatovenerol. Croat.*, **11**, 158–162.
10) Sayes, C. M., Wahi, R., Kurian, P. A., Liu, Y., West, J. L., Ausman, K. D., Warheit, D. B. and Colvin, V. L. (2006) Correlating nanoscale titania structure with toxicity: A cytotoxicity and inflammatory response study with human dermal fibroblasts and human lung epithelial cells. *Toxicol. Sci.*, **92**, 174–185.
11) Guur, J. R., Wang, A. S., Chen C. H. and Jan, K. Y. (2005) Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epitherial cells. *Toxicology*, **213**, 66–73.
12) Ihara, T., Yamamoto, T., Sugamata, M., Okumura, H. and Ueno, Y. (1998) The process of ultrastructural changes from nuclei to apoptotic body. *Virchows Arch.*, **433**, 443–447.
13) Takeda, K., Tsukue, N. and Yoshida, S. (2004) Endocrine-disrupting activity of chemicals in diesel exhaust and diesel exhaust particles. *Environ. Sci.*, **11**, 33–45.
14) Fujimoto, A., Tsukue, N., Watanabe, M., Sugawara, I., Yanagisawa, R., Takano, H., Yoshida, S. and Takeda, K. (2005) Diesel exhaust affects immunological action in the placenta of mice. *Environ. Toxicol.*, **20**, 431–440.
15) Yoshida, S., Ono, N., Tsukue, N., Oshio, S., Umeda, T., Takano, H. and Takeda, K. (2006) In utero exposure to diesel exhaust increased accessory reproductive gland weight and serum testosterone concentration in male mice. *Environ. Sci.*, **13**, 139–147.
16) Ono, N., Oshio, S., Niwata, Y., Yoshida, S., Tsukue, N., Sugawara, I., Takano, H. and Takeda, K. (2007) Prenatal exposure to diesel exhaust impairs mouse spermatogenesis. *Inhal. Toxicol.*, **19**, 275–281.
17) Sugamata, M., Ihara, T., Takano, H., Oshio, S. and Takeda, K. (2006) Maternal diesel exhaust exposure damages newborn murine. *J. Health Sci.*, **52**, 82–84.
18) Sugamata, M., Ihara, T., Sugamata, M. and Takeda, K. (2006) Maternal exposure to diesel exhaust leads to pathological similarity to autism in newborns. *J. Health Sci.*, **52**, 486–488.
19) Kreilgaard, M. (2002) Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Deliv. Rev.*, **54**, 77–98.
20) Tinkle, S. S., Antonini, J. M., Rich, B. A., Roberts, J. R., Salmen, R., DePree, K. and Adkins, E. J. (2003) Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ. Health Perspect.*, **111**, 1202–1208.
21) Takenaka, S., Karg, E., Roth, C., Schulz, H., Ziese-

nis, A., Heinzmann, U., Schramel, P. and Heyder, J. (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. *Environ. Health Perspect.*, **109**, 547–551.

22) Nemmar, A., Hoet, P. H., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M. F., Vanbilloen, H., Mortelmans, L. and Nemery, B. (2002) Passage of inhaled particles into the blood circulation in humans. Circulation. *Circulation*, **105**, 411–414.

23) Meiring, J. J., Borm, P. J., Bagate, K., Semmler, M., Seitz, J., Takenaka, S. and Kreyling, W. G. (2005) The influence of hydrogen peroxide and histamine on lung permeability and translocation of iridium nanoparticles in the isolated perfused rat lung. *Particle and Fibre Toxicology*, **2**, 3.

24) Samet, J. M., DeMarini, D. W. and Malling, H. V. (2004) Do airborne particles induce heritable mutations? *Science*, **304**, 971–972.

25) Lockman, P. R., Oyewumi, M. O., Koziara, J. M., Roder, K. E., Mumper, R. J. and Allen, D. D. (2003) Brain uptake of thiamine-coated nanoparticles. *J. Control. Release*, **93**, 271–282.

26) Kreyling, W. G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdörster, G. and Ziesenis, A. (2002) Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *J. Toxicol. Environ. Health A*, **65**, 1513–1530.

27) Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W. and Cox, C. (2004) Translocation of inhaled ultrafine particles to the brain. *Inhal. Toxicol.*, **16**, 437–445.

28) Warheit, D. B., Brock, W. J., Lee, K. P., Webb, T. R. and Reed, K. L. (2005) Comparative pulmonary toxicity inhalation and instillation studies with different TiO_2 particle formulations: impact of surface treatments on particle toxicity. *Toxicol. Sci.*, **88**, 514–524.

29) Wamer, W. G., Yin, J. J. and Wei, R. R. (1997) Oxidative damage to nucleic acids photosensitized by titanium dioxide. *Free Radic. Biol. Med.*, **23**, 851–858.

30) Renwick, L. C., Donaldson, K. and Clouter, A. (2001) Impairment of alveolar macrophage phagocytosis by ultrafine particles. *Toxicol. Appl. Pharmacol.*, **172**, 119–127,

31) Long, T. C., Saleh, N., Tilton, R. D., Lowry, G. V. and Veronesi, B. (2006) Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. *Environ. Sci. Technol.*, **40**, 43–46.

— Review —

Early Development Origins of Adult Disease Caused by Malnutrition and Environmental Chemical Substances

George Xu,[a, b] Masakazu Umezawa,[a] and Ken Takeda*,[a]

[a]*Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278–8510, Japan and* [b]*Faculty of Arts and Sciences, Harvard College, 1730 Cambridge St., Cambridge, MA 02138, USA*

(Received September 24, 2008)

We observed that maternal exposure to diesel exhaust (DE) and diesel exhaust particles (DEPs) damaged the reproductive and central nervous systems in mice and rats. These observations suggest that impairment of early development induced by maternal exposure to DE and DEP causes several disorders after growing up. To elucidate the effects of maternal exposure to environmental substances, we review here a hypothesis of fetal and early developmental origins of adult disease. Recent studies influenced by Dr. Barker's Thrifty Phenotype Hypothesis have led to advances in understanding how fetal and infant malnutrition can permanently and adversely alter the development of tissues and organs. Several epidemiological surveys in humans have uncovered links between maternal malnutrition and effects on the organs such as the kidney, pancreas, liver, muscles, adipocytes, and the hypothalamic-pituitary-adrenal (HPA) axis. These observations were examples of critical period programming. The idea has been applied to examining possible fetal and early origins of other diseases. Interestingly, many reports showed that similar phenomena were induced by perinatal exposure to airborne environmental pollutants. Studies have shown that maternal DE exposure disrupts reproductive development and damages the central nervous system. In addition, perinatal exposure to tobacco smoke has been linked to several respiratory disorders. These results show that early development is a critical determinant of adult physiology and much care should be taken to ensure the proper environment for fetal development. This idea is especially topical currently, where rapid industrialization in Asia has accelerated changes in environment and increased pollution.

Key words —— thrifty phenotype hypothesis, early development, maternal exposure, diesel exhaust, environmental tobacco smoke, critical period programming

INTRODUCTION

We observed that mice that were maternally exposed to diesel exhaust (DE) and diesel exhaust particles (DEPs) showed signs of damage to the reproductive and central nervous systems. These observations suggest that impairment of early development induced by maternal exposure to DE and DEP causes several disorders after growing up. To elucidate the effects of maternal exposure to environmental substances, we review here a hypothesis of fetal origins of adult disease and its related references.

*To whom correspondence should be addressed: Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641, Yamazaki, Noda, Chiba 278–8510, Japan. Tel.: +81-4-7121-3618; Fax: +81-4-7121-3784; E-mail: takedak@rs.noda.tus.ac.jp

The main theory of fetal origins of adult disease was put forth by Dr. David J.P. Barker in the early 1990's.[1,2] The hypothesis stated that physiological development *in utero* is tailored to the environment that the fetus indirectly senses through the mother. Then, development of certain organs ceases either *in utero* or postnatally and certain features become permanent. If the environment after birth is different from the one sensed by the fetus, these permanent changes can be maladaptive and lead to adult disease. The specific example that Dr. Barker considered is the link between perinatal malnutrition and offspring adult diseases related to metabolic syndrome. He theorized that some cases of adult disease can be attributed to an adverse environment (*e.g.*, malnutrition) during fetal development. This malnutrition then leads to permanent changes in the growth, metabolism, and vasculature of various organs which predisposes the child to adult disease.