抗性を示す不応例に対し、リツキシマブ併用療法の寛解導入としての有効性を非盲検ランダム化比較試験にて検証した。米国ジョンズホプキンス大学、メイヨークリニックなどが中心となり、不応例に対する寛解導入療法におけるリツキシマブの有効性を検証するため、二重盲検ランダム化デザインでの非劣性試験であるRAVE試験（NCT00104299）が行われている。また、ヒト化抗CD20モノクローナル抗体であるオクレリズマブ、完全ヒト型抗CD20モノクローナル抗体であるオファツムマブも欧米にて承認され、血管炎に対する探索試験も検討されつつある。

その他の生物学的製剤の試みとして、ヒトのcytotoxic T-lymphocyte-associated antigen 4（CTLA-4）の細胞外部分と免疫グロブリンIgG1のFc部分の融合タンパク質であるアバタセプト（CTLA4-Ig）が挙げられる。本剤は抗原提示細胞上のCD80／86に結合し、T細胞上のCD28との結合を阻害することでT細胞活性化共刺激を抑制する。アバタセプトのPhase Ⅱ／Ⅲ, proof of conceptのための探索的試験―ABAVAS試験（NCT00048282066）がランダム化二重盲検化、プラセボ-コントロール試験として2007年よりEUVASを中心に7カ国で実施中である。

新たな生物学的製剤の可能性として、動物実験でanti-MPO IgGとLPS（lipopolysaccharide）で誘導された壊死性半月体形成性糸球体腎炎に補体C5a阻害薬を投与し、尿所見と腎組織病変の改善が報告されたことより[15]、補体に対する抗体治療が注目されている。抗C5aモノクローナル抗体であるエクリズマブは発作性夜間血色素尿症の患者の治療として現在治験中であり、血管炎の治療薬としても注目されている。

また、リツキシマブの良好な臨床試験結果から、血管炎の病態におけるB細胞の重要性が注目されている。B細胞の生存、成熟を促すことで、液性免疫の増強に働くB-cell activating factor of the TNF family（BAFF）／a proliferation-inducing ligand（APRIL）抗原系とその負のB細胞受容体であるtransmembrane activator and calcium-modulator and cyclophilin ligand interactor（TACI）などが新たな分子標的として注目されている。

このように基礎研究の発展により臨床応用が拡大され、また、臨床試験の結果から病態が理解され基礎研究の発展につながっており、今後の動向にも注目したい。

（猪原登志子）

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(2)基礎研究

ANCAワークショップでは、基礎研究の領域からANCA血管炎の動物モデル、ANCAの産生メカニズム、リツキシマブの効果発現メカニズムに関連したB細胞の機能、好中球の獲得免疫系への関与、補体系の血管炎への関与、ANCAエピトープ等がトピックスとなった。以下にその概要を紹介する。

①1980年代にANCAが発見されて以来、in vitroにおけるANCAの好中球活性化のメカニズムはかなり明らかにされてきた。しかし、実際にANCAが血管炎のマーカーであるだけでなく病原性があると証明したのは、2002年Chapel HillのXiao ら[1]がMPO欠損マウスを用いて行った研

究である。その後、この動物モデルを使ってANCA血管炎の炎症を規定する因子（補体、PI3キナーゼ、p38や好中球と血管内皮の接着因子など）がin vivoの実験系で次々と報告され、この国際会議でも話題となった。これらの実験系は、ANCA産生より下流の血管炎発症機序を探索するには適しており、新しい治療薬の開発のためにきわめて有用である。

②ANCA産生メカニズムについて、最近オーストリアのKainらのmolecular mimicry（分子模倣）説の報告[2]が話題を集めた。グラム陰性菌のFimHという抗原とヒトLAMP（lysosomal membrane protein）-2がきわめて相同性が高いことに着目した。この細菌感染をきっかけに産生されたFimHに対する抗体が、好中球や血管内皮に発現したLAMP-2に交差反応することにより血管炎を発症するというものである。さらに、壊死性半月体形成性糸球体腎炎の患者は抗LAMP-2抗体の陽性率が高く、その大多数はMPOあるいはPR3-ANCA陽性であることは興味深い。細菌感染をきっかけにANCAが産生されるメカニズムを明らかにし、MPO、PR3に次ぐ第3の重要なANCA抗原を提唱した報告として話題を集めた。

③血管炎の発症メカニズムにおけるneutrophilとB細胞の関与が話題となった。B細胞の表面抗原CD20に対する抗体であるリツキシマブの臨床試験における良好な成績から、ANCA血管炎におけるB細胞の機能異常に注目が集まった。リツキシマブはB細胞を一時的に除去するだけでなく、B細胞の免疫学的性質を正常化し、B細胞が復活した後も免疫学的に寛解状態を維持する。

一方、好中球はANCA血管炎の病理において血管傷害をもたらすエフェクター細胞として位置づけられてきた。従来から宿主の防御の第一線に関わる終末分化細胞として、免疫細胞とは一線を画されてきた感がある[3]。ところが、2003年イタリアのScapiniら[4]により、好中球は血管炎の発症に際してB細胞活性化因子（B lymphocyte stimulator；BLyS）を産生して自己免疫に大きく関与することが報告され、BLySはその受容体を含め新しい生物学的製剤開発の分子標的となっている。その後も、千葉大のHosinoら[5]はMPO-ANCAが好中球を活性化することによりIL-17Aをはじめ IL-6、IL-23を産生して自己免疫性血管炎の進展に重要なTh17細胞を誘導することを報告し、もはや好中球は自然免疫にとどまらず獲得免疫にも深く関与する細胞であるとの認識が、本国際会議においても強調された。

④補体の活性化は血管炎の初期誘導に必須ではないが、炎症を増幅するのにきわめて重要な因子として働く。補体で世界的に有名なDahaが最新知見を盛り込んだreviewを行った。ANCA血管炎の動物モデルではすでにC5aの阻害により血管炎を抑制できることが示されている[6]。さらにヒトのANCA腎炎の傷害糸球体においてもC3dの沈着が顕著であることが示され、補体経路の中でも特にalternative pathwayが活性化されていることが最新の知見として発表された。Dahaは、組織の整合性が破壊された時にproperdinが認識センサーとして機能して補体のalternative pathwayが活性化することを最新のトピックスとして解説した。

⑤MPO-ANCAに病原性があることは前記のように示されてはいるが、実際には血管炎の活動性とANCAの抗体価は比例しない場合も多いことは臨床家のよく知るところである。日本からは、千葉大学の鈴木和男先生らが国際会議シンポジウムで、MPO-ANCA抗体が実際に認識している抗原のうち血管炎の重症度と関連した抗原認識部位をANCAのリスクエピトープとして報告し、本質的かつ秀逸な研究として高い評価を受けた。また、東京医大八王子医療センターの吉田雅治先生は、MPO-ANCAの高親和性と低親和性の差異と血管炎の臨床像との関連について報告し[7]、重症化を予測し早期の治療へと導く指標を与える研究として注目された。

⑥杏林大学の川嶋聡子先生、有

村義宏先生はヒトMPO-ANCA腎炎の腎組織標本を用いて、傷害糸球体の毛細血管壁に細胞外MPOが沈着することを血管内皮マーカーであるCD34とともに染め分け、血管炎初期の内皮障害におけるMPOの重要性を病理学的に示して講演会場の注目を集めた。

⑦東邦大の高橋啓先生はマウスの冠動脈炎モデルを用いた病理学的解析により、我が国に特に多い乳児期血管炎である川崎病のメカニズム解明の重要な報告をした。

⑧大阪・北野病院の武曾恵理先生らはMPA患者の血清を用いて、M-CSF(macrophage-colony stimulating factor)、IL-18、IL-8、MCP-1(monocyte chemotactic protein-1)などを疾患活動性と相関する血清サイトカインとして報告し、臨床応用への大きな示唆を与えた。

⑨Debateは「ANCAが先か?血管炎が先か?」というテーマで、米国Chapel HillのFalk, オランダGroningenのKallenberg, フランス・パリのPhillippe Lesavreが議論を交わした。前者の例としてすでに述べた分子模倣性メカニズムによる細菌感染に伴うANCA産生や、抗PR3抗体陽性WG患者の60%以上が黄色ブドウ球菌のキャリアであることなどが紹介された。一方、後者の例として、急速進行性腎炎の原因検索によりANCA関連腎炎を発見した際、CT所見で無症状の間質性肺炎の陰影を発見することや、抗糸球体基底膜抗体腎炎の症例にMPO-ANCAを同時に検出することなどが指摘された。これらの現象は、組織傷害に続発してANCAが産生されるsecondary autoimmunizationの例として紹介された。

⑩以上のようにANCA国際会議では、免疫学者や細菌学者、遺伝学者、呼吸器病学、腎臓病学および膠原病学の専門家など、多領域の専門家が集結して議論が交わされた。ANCA血管炎が多臓器疾患であるだけでなく、その病態メカニズムの解明および治療法の確立のためには、世界の基礎および臨床家の総力を結集する必要があることを再認識させられた。

(平橋淳一)

□■□文 献□■□

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まとめ

血管炎の概念・分類基準に関して、国際的な広がりの中、欧米はもとより、遺伝子背景や環境、生活習慣の異なる地域との比較が重要である。日英での議論の中で、血管炎の違いが浮かび上がり、厚労省ヒューマンサイエンス橋本博史班で本格疫学調査が開始された。その調査と並行して日英での基準に関する討論が始まった。日本の血管炎と欧米の血管炎の疫学調査から、臨床症状・ANCA型別の頻度など相違点が多いことが報告され、この差異の原因には、①疾患概念、②診断方法、③分類/診断基準の相違などの影響などが考えられた。また、診断方法の一つのMPO-ANCA測定のELISA(enzyme-linked immunosorbent assay)キットが日本と欧米で相違がないことが最近明らかにされた[1]。**表2**にこれまで筆者らが関わってきた国際会議の背景を示した。

しかし、その打ち合わせや会議だけでは、その違いなどを説明するには壁があった。一方、これまでの診断基準や分類基準はだいぶ古くなっており、見直しをすべきとの意見が欧州を中心として広がり、EULAR(the European league against rheumatism, 欧州リウマチ学会)/ACR(American college of rheumatology, 米国リウマチ学会)を中心に見直しの討論が開始された。2008年3月と12月にチューリッヒにおいて、EULAR/ACRによる概念・診断名・分類基準・診断基準などの会議(Development EULAR/ACR endorsed of points to consider in the diagnosis, definition and classification of systemic vasculitis EULAR House, Zürich,

表2 疫学調査と新基準に関する会議

関連調査会議：日英を中心として		
2004年	英国・ケンブリッジ，ノービッチ	日英の血管炎の相違についての議論
2004年～2007年	国内調査開始	欧米の研究者による日本国内の調査：沖縄，宮崎，仙台，盛岡，旭川（HS橋本班）
2004年	英国・ケンブリッジ	日英の血管炎の相違についての議論
2005年	東京，沖縄，宮崎	英国の研究者による日本国内の調査
2005年	東京	Watts, Scottの提案のEMEAのアイディアについての討論
2005年	独国・ハイデルベルク	日英の血管炎の相違についての議論：12回ANCA会議出席者
2006年	東京，仙台，盛岡	英国の研究者による日本国内の調査
2007年	東京（英国大使館），旭川	欧米の研究者による肺腎血管炎の討論および日本国内の調査
EULAR／ACRの血管炎：New classificationに関する会議		
2006年	スイス・チュリッヒ	New classficationに関する論文の調査会議：予備会議
2008年	スイス・チュリッヒ	New classficationに関する討論：運営委員
2008年	スイス・チュリッヒ	New classficationに関する提案会議：運営委員
2009年	スウェーデン・ルンド	New classficationに関する提案など：ANCA会議に出席した運営委員
2009年		欧州・米国・日本の3地域の施設から検証のための登録
2009年	米国・フィラデルフィア	New classficationに関する状況報告と予算申請の提案など

March 3rd～4th, 2008）が開催された（表2）。日本と欧米の血管炎の疫学・臨床症状の相違を考えるには、各種血管炎の基本的概念・定義・分類法などの国際的な基準を理解し、比較検討しなければならないが、まだ新基準は確立していない。

検討されてきた骨子は以下のようである。ACRの分類基準とCHCC定義に限界があり、多くの機会で、「診断基準」として、専門家ではない医師や専門に近い医師にも誤って使用される。

取り上げられた項目は、(1)血管炎vs主として非炎症性血管障害、(2)抗リン脂質抗体症候群と感染症、(3)原発性と続発性血管炎、(4)「傷害される血管の大きさ」で分類される群に〝傷害される血管の口径に特異性がない群〟が加えられたこと、(5)〝分類未確定〟（unclassified）鑑別ができない状態、(6)診断の信頼性・確実性を以下の表現で定義した：(7)二次性血管炎は、感染、薬剤、悪性腫瘍、膠原病の亜型にさらに分類したことであり、(8)最終的には、すべての状態は個別に区別された分類樹（〝classification tree〟）に分類された。

文献の広範な検討と専門家のコンセンサスにより、最新の定義や基準の中で基本的に改善すべきことが明らかになった。多施設が参加する更新された定義の検証を行い、血管炎の新しい分類基準を作成すべきであると考えられた。この議論はまとめられて、論文として公開される予定である。さらに、この議論から抽出された提案は、欧州・米国・日本の関連施設の登録により検証される予定である。

なお、Wattsらによって提唱されたEMEA（european medicines agency）アルゴリズムは、本classificationとは区別されるものである。

（鈴木和男）

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END-STAGE RENAL DISEASE

GFR and albuminuria as predictors: two is better than one

Enyu Imai

Identifying patients at risk of end-stage renal disease relying only on measurement of both glomerular filtration rate and albuminuria could greatly decrease the number of patients flagged for renal surveillance without increasing the risk of overlooking high-risk individuals.

As prevalence and incidence of end-stage renal disease (ESRD) increases worldwide, health-care expenditure to treat ESRD and its complications, including cardiovascular diseases, is ballooning. Early identification of patients at risk for developing ESRD could offer an opportunity to reduce incidence of the disease and the cost associated with preventing and treating it. Stein Hallan and colleagues[1] demonstrated for the first time that a combination of estimated glomerular filtration rate (eGFR) and albuminuria is the best prognostic predictor of chronic kidney disease (CKD) progression to ESRD. The researchers introduce a classification system that could lead to referral for risk of ESRD of only ~1% of the general population. This patient subset would still include about two-thirds of those expected to progress to ESRD.

The Kidney Disease Outcomes Quality Initiatives (K/DOQI) of the National Kidney Foundation defines CKD as a clinical condition lasting more than 3 months associated with kidney damage—assessed by blood or urine analysis or imaging tests—or reduction of eGFR to less than 60 ml/min/1.73 m^2.[2] Screening for CKD can be done by measuring serum creatinine levels and by dip-stick testing for proteinuria. However, the simplicity of CKD definition might result in over-reliance on the screening results and overtreatment of patients, particularly those with eGFR 45–59 ml/min/1.73 m^2.

A major incentive to categorize patients according to CKD stage is to stratify risk of adverse outcomes. The majority of individuals with CKD have eGFR just below 60 ml/min/1.73 m^2, but ESRD risk level across this patient subset differs widely. In fact, CKD stage 3 (GFR 30–59 ml/min/1.73 m^2) comprises 45–80% of the CKD population and 7–10% of general population.[3–5] In individuals with eGFR 50–59 ml/min/1.73 m^2 the risk of progression to ESRD was shown to be low, particularly among elderly patients.[6,7] In addition, most patients with CKD stage 3 do not have proteinuria, an independent and strong risk factor for ESRD. According to the third National Health And Nutrition Examination Survey (NHANES III 1999–2004), 92% of patients with stage 3 CKD have normal urine and only 7.4% and 0.8% have microproteinuria or macroproteinuria, respectively. In the Japanese annual health screening program, CKD stage 3 comprises 10.4% of the general population, and 92.3% of patients in this category do not have proteinuria.[4] Patients without proteinuria could be at a considerably reduced risk of ESRD compared with those with proteinuria. A 17-year follow-up study enrolling 106,177 individuals from the general population of Okinawa clearly shows that 1+ proteinuria measured by dipstick is a significant risk factor for progression to ESRD compared with trace or absence of proteinuria.[8] In a Japanese study, eGFR was measured twice over 10 years in each of 120,727 participants recruited from the general population. Presence of proteinuria was found to more than double the rate of GFR decline.[6]

Proteinuria is a strong and independent risk factor for cardiovascular disease as well as for ESRD.[9] An increase in proteinuria results in a proportional increase in cardiovascular events. Patients with proteinuria and stage 1 (>90 ml/min/1.73 m^2) or 2 (60–89 ml/min/1.73 m^2) CKD might have a worse cardiovascular prognosis than patients with stage 3 CKD without proteinuria.

Hallan *et al.*[1] analyzed data on 65,589 adults from the Norwegian general population who participated in the Nord-Trøndelag Health 2 (HUNT 2) study. After a mean follow-up of 10.3 years, 124 participants progressed to ESRD. The hazard ratio (HR) of progression to ESRD increased with the reduction of eGFR as well as with the increase of albuminuria, as measured by albumin:creatinine ratio (ACR). Furthermore, the HR of the best clinical model for ESRD progression, which was composed of age, gender, physical activity, diabetes, systolic blood pressure, antihypertensives and HDL cholesterol, was not significant after accounting for eGFR and ACR. If individuals with eGFR ≥60 ml/min/1.73 m^2 and normal ACR are taken as reference, the adjusted HR of individuals with normal (≥60 ml/min/1.73 m^2) eGFR was 27.3 and 196.3 for participants with microalbuminuria and macroalbuminuria, respectively. The adjusted HR of individuals with normal ACR was 23.4, 51.9, and 368.7 in association with eGFR 45–59 ml/min/1.73 m^2, 30–44 ml/min/1.73 m^2, and 15–29 ml/min/ 1.73 m^2, respectively. The HR of ESRD progression of patients with eGFR 15–29 ml/min/1.73 m^2 and macroproteinuria was 4,146.0.

The researchers also analyzed sensitivity and specificity of various indicators by the receiver operating curve (ROC) analysis. The partial area under the ROC is

Box 1 | End-stage renal disease risk stratification in developing countries

- Chronic glomerulonephritis is the leading cause of end-stage renal disease (ESRD) in many developing countries
- Chronic glomerulonephritis is initially associated with normal renal function in the context of hematuria, proteinuria or both
- Risk of progression to ESRD should be stratified by measuring albuminuria
- Given the high cost of measuring albumin:creatinine ratio in urine, patients with chronic kidney disease stage 3 should be screened for albuminuria by dipstick urinalysis
- Patients with chronic kidney disease stage 3 and 1+ proteinuria should be considered at high risk of ESRD

a measure of the proportion of patients classified correctly in terms of progression to ESRD. The partial area under the clinically relevant part of ROC (false-positive rates 0.0–0.10) was 0.704 for the best clinical model alone, 0.786 for ACR alone, 0.821 for eGFR alone, and 0.844 for the combination of ACR and eGFR. Referring all study participants with CKD stage 3–4 to a specialist would have meant the referral of 4.7% of the entire cohort and 69.4% of patients who would actually progress to ESRD. Consequently, the number of patients not progressing to ESRD referred for every case of actual ESRD (NNTF) would have been 35.4. On the other hand, patients at moderate or high risk of ESRD, as measured by a combination of eGFR and ACR, comprised 1.4% of the study cohort and 65.6% of patients eventually progressing to ESRD (NNTF = 11.4). Clearly, the combination of eGFR and albuminuria improves ESRD risk stratification by considerably reducing the number of potential referrals (and associated cost) while still highlighting the majority of patients who will progress to ESRD. However, the screening system proposed by Hallan and colleagues needs to be validated in a different participant cohort.

Screening for CKD has been mainly focused on disease caused by lifestyle. Although this approach might be reasonable for developed countries with high prevalence of hypertension and diabetes, in many developing countries chronic glomerulonephritis is the leading cause of ESRD.[10] In its early phase, chronic glomerulonephritis is generally associated with urinary abnormality, including hematuria or proteinuria, in the context of normal renal function. ACR screening of patients with CKD stage 3 could help identify high-risk patients and decrease the incidence of chronic-glomerulonephritis-associated ESRD. However, given that ACR measurements are expensive and time consuming, dipstick urinalysis should probably be used to estimate ESRD risk (Box 1).

Risk stratification by two-dimensional information on eGFR and proteinuria can improve the prediction of progression to ESRD; revision of CKD classification by including albuminuria, particular in patients currently categorized as having stage 3 CKD, could resolve the argument of whether CKD staging is a practical approach to decreasing incidence and prevalence of ESRD.

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Competing interests
The author declares no competing interests.

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TRANSPLANTATION

Pregnancy after kidney donation: more questions than answers

Michelle A. Josephson

Two recent reports in the *American Journal of Transplantation* focus on the maternal and fetal outcomes of pregnancies in kidney donors and provide tantalizing, if somewhat worrisome, observations. The findings also leave us with several important unanswered questions.

Marginal increases in blood pressure and diminished kidney function have been noted in kidney donors.[1,2] As a result of these observations, Reisæter *et al.* were concerned that female kidney donors might be at risk for hypertensive disorders of pregnancy. To test their hypothesis, the authors analyzed data from the Medical Birth Registry of Norway, a database that contains information from all pregnancies in Norway that were viable at 16 weeks, from 1967 onwards.[3] The Norwegian Renal Registry provided kidney transplant records of all kidney transplantations performed in Norway between 1967 and 2002. These two data sources were linked and 326 donors were identified, with 620 pregnancies registered before donation and 106 pregnancies registered after donation. Pregnancies before donation served as controls as did 21,511

難治性ネフローゼ症候群

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Refractory nephrotic syndrome.
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Ⅰ．難治性ネフローゼ症候群の定義

ネフローゼ症候群は，1973年に厚生省特定疾患ネフローゼ症候群調査研究班にて定義されている[1]。尿蛋白3.5g/日以上を持続し，かつ血清総蛋白6.0g/dL以下または，血清アルブミン値3.0g/dL以下であることが，ネフローゼ症候群には必須項目である。必須項目ではないが，浮腫は重要な所見である。脂質異常症は多くの症例に認められ，診断基準にあげられているが，参考条件で必須項目ではない。ネフローゼ症候群が治療により改善することを寛解というが，同じくネフローゼ症候群調査研究班にて1974年に決められた治療効果判定基準の定義を表に示す。この寛解の定義は日本と米国では異なっている。わが国の完全寛解は蛋白尿の消失であり，欧米でのremissionは0.3g/日(0.2g/日)未満になることである。また，1日の尿蛋白が1.0g/日未満に低下すると予後が明らかに改善するため，1.0g/日未満になることを不完全寛解Ⅰ型と呼び，尿蛋白が減少するも，1.0g/日以上を不完全寛解Ⅱ型と区別している。欧米ではpartial remissionといい，3.5g/日未満になるか，場合によっては尿蛋白量が50%以下になった場合と定義される[2]。

日本における難治性ネフローゼ症候群は，「6ヵ月以上種々の治療(ステロイドと免疫抑制薬は必須)をして治療しても完全寛解または不完全寛解Ⅰ型に至らない場合」と定義する。実際の臨床の場では，ステロイドで治療を開始して，2週目または4週目に有効性を判定することが多く，6ヵ月の間に治療法が変更されることが通常である。1.0mg/kg体重のステロイドによる4週間の治療が有効でない場合には，ステロイド抵抗性と定義する。早期発見，早期治療を目指す日本と異なって，欧米においては，長期予後(腎死，死亡)にて治療の有効性が判断されていることもあり，自然寛解する可能性のある，尿蛋白が4.0g/日未満の膜性腎症は

表1．ネフローゼ症候群の治療判定基準の定義

	欧米	日本
完全寛解	尿蛋白<0.3g/日(<0.2g/日)	尿蛋白消失，血清蛋白の改善，および他の諸症状の消失がみられるもの
不完全寛解	尿蛋白<3.5g/日かつ尿蛋白の50%以上の減少	Ⅰ型：血清蛋白の正常化と臨床症状の消失が認められるが，尿蛋白が持続するもの。尿蛋白<1.0g/日 Ⅱ型：臨床症状は好転するが，不完全寛解Ⅰ型に該当しないもの
無効	尿蛋白≧3.5g/日	治療に全く反応しないもの

6ヵ月間は利尿薬，ACE-I，ARB，スタチンで治療し，ステロイドや免疫抑制薬を使用しないで経過をみることも推奨されている[3]。

Ⅱ．原因疾患の特徴と治療

わが国では2007年に日本腎臓学会腎病理標準化委員会が中心になって，腎生検患者のレジストリーを構築した。現在，6,000例を超える登録が行われている。

2007年から2009年の登録でネフローゼ症候群はこのなかの約20%を占める。成人においては膜性腎症が最も多く，微小変化群，巣状糸球体硬化症がこれに続く。

1．膜性腎症

膜性腎症は成人ネフローゼ症候群のなかで26%を占め，最も多い。

膜性腎症は約20～30%が無治療でも寛解するといわれている。腎生存率は5年で95%，10年で89%，15年で80%，20年で59%である。また，欧米の腎生存率は日本よりも悪く，10年で20～30%が腎不全をきたすといわれる。

膜性腎症は，ポドサイトの下，基底膜上皮側に免疫複合体の沈着がみられ，基底膜が肥厚する疾患である。免疫組織学的には，顆粒状のIgG，C3が沈着し，電顕的にはdense depositとして認められる。膜性腎症の病期分類は電子顕微鏡によるEhrenreich-Churg分類でステージⅠ～Ⅳまでに分類され，ⅠからⅣに向かって進行する。Yoshimotoら[4]は，単一時相の沈着物からなる均一型(homogeneous)とⅠ～Ⅳの各層がみられるのを，混合型(heterogeneous)と分類し，予後が異なることを報告している。混合型は均一型と比較して，寛解率が低い。

2009年にポドサイトに存在するPhophlipase A2受容体(PLA2R)に対する自己抗体ができ，*in situ*で免疫複合体を形成することによる膜性腎症が約70%を占める原因となることが報告された[5]。このPLA2R抗体は患者の血清中に存在し，寛解により消失することも報告されている。PLA2R抗体が難治性と関連するかどうかは不明である。

膜性腎症の治療はステロイド単独療法でも有効であるという，わが国での報告もあり[1]，通常ステロイド単独の治療がまず4週間程度開始される。ステロイドの反応性をみて，効果がない場合に免疫抑制薬が追加される。欧米のランダム化比較試験の検討では，ステロイドの単独使用は寛解導入に有効ではないことが示されており[6]，通常免疫抑制薬との併用療法が行われている。現在わが国で，難治性ネフローゼ症候群に対して保険適応可能なものは，シクロスポリンとミゾリビンであり，4週間ステロイドを投与しても尿蛋白が不完全寛解Ⅰ型以下にならない症例に対して，ステロイドとの併用の有効性に関するランダム化比較試験が行われている。

ステロイドに免疫抑制薬を追加した治療を行った後も，尿蛋白が1.0g/日以上が続く場合は難治性ネフローゼ症候群と定義される。膜性腎症の難治性ネフローゼ症候群の場合(特に不完全寛解Ⅱ型)は自然寛解が起こることを期待して，ステロイド20～10mgに減量して2～3年継続する場合もある。また，浮腫が利尿薬でコントロール可能であれば，ステロイドが無効であると判断した場合には中止して，経過をみる場合もある。無効例についてはステロイドとシクロスポリンとミゾリビンの3剤併用も行われる。欧米では，リツキシマブの試験的使用が行われており，尿蛋白の約50%程度の改善が報告されている[7]。

2．巣状糸球体硬化症

巣状糸球体硬化症(Focal Segmental Glomerulosclerosis；FSGS)は，ネフローゼ症候群をきたす原発性糸球体腎炎の6%を占める。

FSGSには自然寛解はなく，腎生存率は5年で85.3%，10年で70.9%，15年で60.9%，20年で43.5%である[1]。

典型的には髄質近傍の糸球体の一部に部分的な硬化病変が認められるものである。硬化した糸球体以外は微小変化群ネフローゼとよく似ており，糸球体のサイズが大きいことと，ポドサイ

トの側突起の融合がみられる。蛍光抗体は陰性のこともあるが，IgMが染色されることもある。

最近，D'Agatiらによって，FSGSのvariantの分類が発表され，病理組織と予後に関する評価がなされている[8]。病理分類のtip lesionは予後がよいといわれ，collapsingは予後が悪いといわれている。臨床的には尿沈渣で，赤血球や顆粒球円柱がみられること，尿蛋白の選択性が低下することなどの特徴があり，鑑別は可能である。

治療により完全寛解または不完全寛解Ⅰ型に至ったFSGSの予後は良好であるため，積極的な治療が必要である。ステロイド単独療法の有効性は認められているものの，寛解に導入できるまでの時間がかかることがわかっている[9]。4ヵ月以上のステロイド治療が60%の症例で必要である。また，シクロスポリンとの併用療法で有効性が認められており，推奨されている。ステロイドを減量できる治療を考えるべきである。

ステロイド抵抗性の症例で，高コレステロール血症を合併している場合には，LDLアフェレーシスを行う。

FSGSの再発の治療には，シクロスポリンとステロイドの併用が有効である[10]。

FSGSの場合，ステロイド抵抗性を示した場合，多剤での治療に反応しない場合には，遺伝性疾患を常に疑う必要があり，家族性に発症していないか確認する[11]。A-actinin4, podocin, WT-1, NIIY9異常で起こることが知られている。

3．微小変化群ネフローゼ症候群

微小変化群ネフローゼ症候群(Minimal Change Nephrotic Syndrome；MCNS)は，成人のネフローゼ症候群の16%を占める。最近，高齢者のMCNSで，治療反応性が悪く，免疫抑制薬を使用せざるを得ない症例が増えている。これらの症例は生命予後が悪く，ステロイド，免疫抑制薬の使用により，感染症にて死亡するケースがみられる。

病理組織学的には，光顕所見は正常で，電子顕微鏡で側突起の融合がみられる。

上気道感染症に続いて起こるケースがある。尿蛋白の選択性は高い。

尿中に排泄されるIgGは少ないにもかかわらず，血中IgGは低下している症例が多い。

ステロイドに対する反応はよく，2週間以内に完全寛解に至る。完全寛解率は90%以上である。しかし，頻回に再発を繰り返す症例が約30%存在する。

難治性ネフローゼ症候群は，ステロイドパルス療法も考慮する。MCNSの病因が，リンパ球が分泌する分子であると考えられており，ステロイドパルス療法は直接リンパ球を減らすため，より強力にT細胞を抑制し，蛋白尿を減らすことができる。

頻回再発型の治療はステロイドと免疫抑制薬をいかに減量，中止するかである。実際は多くの症例で，中止できず少量のステロイドにシクロスポリンを併用する治療が継続して行われる。

4．膜性増殖性糸球体腎炎

膜性増殖性糸球体腎炎(Membranaoproliferative Glomerulonephritis；MPGN)は，成人ネフローゼ症候群の5%を占める。多くは二次性のMPGNである。

メサンギウム細胞の増殖と糸球体係蹄壁の肥厚，糸球体の分葉化，糸球体係蹄内腔の狭小化がみられる。メサンギウム間入による基底膜二重化も特徴的である。蛍光抗体では，C3，IgG，IgMなどが染色する。

C型肝炎に合併するものが多かったが，全身性エリテマトーデス，関節リウマチ，感染症，溶血性尿毒症症候群，抗リン脂質抗体症候群，クリオグロブリン血症などで起こる。原疾患の鑑別診断が重要である。

通常，原疾患の治療を優先する。

治療は，確立されていない。難治性ネフローゼ症候群では，ステロイドパルス療法を含めた，シクロフォスファミド，シクロスポリンなどの併用療法が行われる。

Ⅲ．合併症・治療による副作用

ネフローゼ症候群の合併症には凝固能亢進による血栓，悪性腫瘍，感染症などがある。大量の尿蛋白が出ている状態は血清免疫グロブリンの減少がみられ，ステロイド，免疫抑制薬を使用するため，易感染性の状態にある。悪性腫瘍は厚生労働省の調査で，3.4%にみられている[1]。膜性腎症の抗原が癌細胞に由来することも報告され[12]ているが，ネフローゼ症候群自体に悪性腫瘍との関連があることが指摘されている。MCNSとHodgkin病，膜性腎症と肺，消化器系悪性腫瘍との関連が報告されている。凝固因子の産生過剰，血管内脱水などにより血栓症は起こりやすい状態にあり，1.1%の合併率が報告されている。予防的にヘパリン，ワルファリンの使用も行われる。腎静脈血栓症の合併は尿蛋白が増加する。感染症は1.9%にみられ，肺炎が最も

多い。

難治性ネフローゼ症候群は長期間ステロイドや免疫抑制薬を使用するため，薬剤による副作用が増加し，6.1%にみられる。ステロイドによる糖尿病発症，精神病，大腿骨頭壊死，緑内障，白内障，胃潰瘍，免疫抑制薬による骨髄抑制，性腺抑制，出血性膀胱炎，間質性肺炎などに注意を要する。

おわりに

ネフローゼ症候群の実態調査は1993年に行われ，追跡結果も含めて2002年に報告されて以来，調査は行われていないため，2008年度の進行性腎障害調査研究班で腎臓学会の腎臓病総合レジストリー・腎生検レジストリーに参加する形で日本ネフローゼ症候群コホート研究(JNSCS)を構築した。このレジストリーより，原因疾患，寛解率，寛解導入に使用する薬剤，その有効性などがわかると思われる。JNSCSへの登録が進むことが鍵となるので，積極的な参加をお願いしたい。

文　献

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Amelioration of Diabetic Nephropathy in OLETF Rats by Prostaglandin I_2 Analog, Beraprost Sodium

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Key Words
Prostaglandin I_2 · Beraprost sodium · Diabetic nephropathy · Otsuka Long-Evans Tokushima Fatty rat

Abstract
Background: Strict control of blood glucose and blood pressure levels sometimes fails to delay the development of diabetic nephropathy, and an effective therapy is not yet available. The present study aimed to examine whether the prostaglandin I_2 analog beraprost sodium (BPS) ameliorates diabetic nephropathy in Otsuka Long-Evans Tokushima Fatty (OLETF) rat. ***Method:*** Fifty-week-old OLETF rats were divided into three groups according to treatment; 400 μg/kg body weight (BW) BPS, 200 μg/kg BW BPS, and 0.9% saline administration. Kidney histology, index of glomerulosclerosis, and glomerular volume were determined, and urine and serum chemistry were assessed. ***Results:*** The values for urine protein excretion and serum blood urea nitrogen in BPS-treated rats were significantly lower than those in untreated rats. In rats treated with 400 μg/kg BW BPS, neither sclerotic changes nor inflammatory cell infiltration were observed. Index of glomerulosclerosis and glomerular volume were also significantly reduced compared with untreated rats. Intriguingly, BPS reduced the level of serum triglyceride. In the glomerulus of treated rats, advanced glycation end product formation and macrophage influx were suppressed in a dose-dependent manner. ***Conclusion:*** These findings indicate that BPS has a therapeutic effect on diabetic nephropathy in the OLETF rat, which suggests a potential application of this drug in the treatment of human diabetic nephropathy.

Introduction

The population of diabetic patients is growing significantly, and diabetic nephropathy is a leading cause of end-stage renal disease in Japan [1]. Strict control of blood glucose and blood pressure (BP) levels sometimes fails to delay the development of diabetic nephropathy, and an effective therapy for diabetic nephropathy is not yet available. It has been suggested that glomerular hyperfiltration is strongly involved in the progression of incipient diabetic nephropathy, and the main mechanism is inappropriate dilatation of afferent arterioles, which may induce glomerular hyperfiltration and hypertrophy followed by thickening of the glomerular basement membrane and accumulation of mesangial matrix [2, 3]. Prostaglandin I_2 (PGI_2) is known to have a relaxant action on vascular smooth muscle [4], and inhibitory ac-

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tions on platelet aggregation [5] and neutrophil function [6]. In fact, prostaglandin was shown to alleviate angiotensin II-induced constriction of efferent glomerular arterioles in vitro [7]. Recently, the PGI_2 analogue beraprost sodium (BPS) was shown to reduce urinary albumin (Alb) excretion in streptozotocin (STZ)-induced diabetic rat [8], and cause a decrease in urinary protein in human type 2 diabetes mellitus [9]. The efficacy of long-term 24-month administration of BPS for reduction of albuminuria in patients with incipient diabetic nephropathy was demonstrated in clinical studies. In this study, we examined the clinical usefulness of BPS for the overt diabetic nephropathy in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, and confirmed improvement by histological analysis of the kidneys.

Materials and Methods

Animals

Male OLETF rats, a model of spontaneous non-insulin-dependent diabetes mellitus, and male Long-Evans Tokushima Otsuka (LETO) rats, the nondiabetic control model of OLETF rats, were kindly supplied by Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Rats were maintained in the Laboratory of Animal Experiments at Fukuoka University. The present experiments were initially reviewed and approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiologic Society.

Drug

The stable PGI_2 analogue, beraprost (sodium-2,3,3a,8b-tetrahydro-2-hydroxy-1-1-[(E)-(3S)-3-hydroxy-4-methyl-1-octen-6-ynyl]-1H-cyclopentabenzofuran-5-butyrate) was provided by Toray Industries, Inc. (Tokyo, Japan).

Experimental Protocol and Laboratory Assessments

Fifty-week-old male OLETF and LETO rats were divided into 3 groups according to the treatment type: group H, 400 μg/kg body weight (BW; OLETF n = 10, LETO n = 7); group L, 200 μg/kg BW (OLETF n = 7, LETO n = 5), and group C, equivalent volume of 0.9% saline (OLETF n = 10, LETO n = 7). Every 4 weeks, the solutions were subcutaneously administered using an osmotic pump (ALZA Co., Palo Alto, Calif., USA). Urinary protein, blood urea nitrogen (BUN), serum creatinine (Cr), plasma total cholesterol (TC), plasma triglyceride (TG), serum total protein (TP), serum Alb and blood glucose were assessed every 4 weeks. Plasma lipoproteins were analyzed by an online dual enzymatic method for simultaneous quantification of cholesterol and TGs by high-performance liquid chromatography (HPLC) as previously described [10, 11].

Histopathology of the Kidney and Liver

Rats were sacrificed at 16 weeks after the beginning of administration. The kidneys and livers were fixed in cold 95% ethanol or 10% formaldehyde for 24 h and embedded in paraffin. Sections (2–3 μm) were treated with periodic acid-Schiff and Sudan-IV staining [12]. The severity of sclerosis in the glomerulus was evaluated in a blind manner by histological examination of the sectioned kidneys, and the results were expressed as an index of glomerulosclerosis (IGS). The glomerular pathology was carried out by assessing 50 glomerular cross-sections per kidney, and the degree of sclerosis in each glomerulus was subjectively graded on a scale of 0–4: grade 0, no change; grade 1, sclerosis area less than or equal to 1/4 of glomerulus or distinct adhesion present between capillary tuft and Bowman's capsule; grade 2, sclerosis of 1/4 to 1/2 total glomerular area; grade 3, sclerosis of more than 1/2 glomerulus but not global, and grade 4, global sclerosis. IGS was calculated using the following formula:

$$IGS = \frac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4}{N0 + N1 + N2 + N3 + N4}$$

where N is the number of glomeruli in each grade of sclerosis.

Glomerular volume (GV) was determined by the mean glomerular diameter, which was calculated from the value of diameters of 50 glomeruli for each kidney specimen (×400).

Immunopathological Studies of the Kidneys

After deparaffinization in xylene and ethanol, and washing in phosphate-buffered saline (PBS), the paraffin-embedded sections were incubated with mouse anti-rat advanced glycation end products (AGEs) Ab (Trans Genic Inc., Kumamoto, Japan), mouse anti-rat CD68 Ab (Hycult biotechnology, Uden, The Netherlands), anti-rat endothelial NO synthase (eNOS) Ab (Abcam Inc., Cambridge, Mass., USA), and neuronal NO synthase (nNOS) Ab (American Research Products, Inc., Belmont, Mass., USA) at the concentration of 1 μg IgG/ml PBS including 1% bovine serum Alb. Staining was performed using anti-mouse IgG-HRP-labeled polymer (DakoCytomation Inc, Carpinteria, Calif., USA). The magnitude of immunostaining for AGE was quantitated using computer-assisted image analysis, as previously described [13, 14]. The proportion of the brown peroxidase-stained area for glomerulus, and the numbers of cells stained with mouse anti-rat CD68 Ab were quantitated on 50 glomeruli per animal. They were expressed as the mean ± SE per glomerular cross-section.

Real-Time Quantitative PCR

We assessed the transcription level of the monocyte chemoattractant protein-1 (MCP-1) gene *(Ccl2)* and angiotensin II type I receptor gene *(Agtr1)* relative to the levels of actin in the kidney cortex. Reverse transcription reactions and TaqMan PCRs were performed in accordance with the manufacturer's instructions (Applied Biosystems Japan, Tokyo, Japan). Sequence-specific amplification was detected with an increased fluorescence signal of FAM during the amplification cycles, using an ABI Prism 7500 sequence detection system (Perkin Elmer Japan, Yokohama, Japan). Oligonucleotide primers and probes were designed using the Primer Express program (Applied Biosystems Japan) and synthesized: Rn01456716-g1 for *Ccl2*, Rn01435427-m1 for *Agtr1*.

Statistical Analysis

Quantitative data were given as the mean value ± SE. ANOVA, followed by Fisher's method (StatView version 5.0), was performed to analyze the differences between groups. A p value of less than 0.05 was considered statistically significant.

Results

Laboratory Assessment Data

In OLETF rats at 4 weeks after the start of drug administration, urine protein excretion in the BPS-treated groups was significantly decreased compared to the control group: group H, 101.7 ± 15.9; group L, 122.7 ± 23.3, and group C, 215.3 ± 33.5 mg/day (group H vs. C, $p < 0.001$; group L vs. C, $p < 0.05$). The urine protein values in group C were always more than 200 mg/day 4 weeks after the beginning of the administration. On the other hand, those of group L and H at 12 weeks after the beginning of the administration were still lower than those before administration. In total, there was a significant difference between group H and C ($p < 0.05$; table 1). The BUN value in group H was also significantly lower than in group C. Although the levels of Alb in groups L and H were significantly higher than those in group C (group H vs. C, $p < 0.05$; group L vs. C, $p < 0.05$), the level of TP in group H was significantly higher than that in group C. There were no significant differences in the levels of Cr and blood glucose.

Glomerular Volume

GVs of groups H, L, and C in OLETF rats were 4.57 ± 3.81, 6.14 ± 2.33, and 9.86 ± 8.63 ($\times 10^4$ μm^3), respectively. There were significant differences in GV between BPS-treated and untreated groups (group H vs. C, $p < 0.0001$; group L vs. C, $p < 0.001$). GVs of LETO rats were almost the same in the three groups (fig. 1a).

Index of Glomerulosclerosis

IGS of group H, L, and C in OLETF rats were 0.80 ± 0.14, 1.28 ± 0.20, and 1.91 ± 0.17, respectively. There were significant differences in IGS between BPS-treated and untreated groups (group H vs. C, $p < 0.01$; group L vs. C, $p < 0.05$; fig. 1b). Histological analysis showed that in group C, sclerotic change in the glomerulus was prominent and inflammatory cell infiltration was notable in the interstitium. On the other hand, the histological findings in group H were almost normal. Glomerulosclerosis was hardly seen in the LETO rats (fig. 1c).

Immunohistological Studies of the Kidneys in OLETF Rats

Reducing sugars, including glucose, fructose and trioses, can react nonenzymatically with the amino groups of proteins to form reversible Schiff bases and subsequently Amadori products. These early glycation products undergo further complex reactions such as rearrangement, dehydration, and condensation to become irreversibly cross-linked heterogeneous fluorescent derivatives, termed advanced glycation end products (AGEs) [15, 16]. The area of AGEs in the glomerulus of BPS-treated rats was apparently reduced compared to untreated rats. The proportions of AGEs area for glomerulus in group C, group L and group H were 12.94 ± 2.04, 7.86 ± 1.87, and 2.29 ± 0.22, respectively (group H vs. C, $p < 0.001$; group L vs. C, $p < 0.01$; fig. 1d). Intraglomerular macrophages were detected by anti-CD68 Ab. Macrophage influx and foamy change of macrophages were found in some glomeruli of untreated rats (fig. 1d, arrow), while in BPS treated rats a few macrophage influx was observed. The numbers of intraglomerular macrophages in groups C, L and H were 3.65 ± 0.19, 2.42 ± 0.18, and 1.72 ± 0.1, respectively (group H vs. C, $p < 0.001$; group L vs. C, $p < 0.01$).

Reduction of Ccl2 and Agtr1 mRNA Expression in the Kidney of BPS-Treated Rats

The RNA expressions of *Ccl2* and *Agtr1* were reduced in the kidney cortex of BPS-treated rats compared with the untreated rats; relative abundance of *Ccl2* mRNA normalized by β-actin in groups C, L, and H was 2.65 ± 0.90, 1.22 ± 0.67, and 0.65 ± 0.45, respectively (group H vs. C, $p < 0.05$). The abundance of *Agtr1* mRNA expressions was 0.17 ± 0.04, 0.09 ± 0.03, and 0.04 ± 0.04, respectively (group H vs. C, $p < 0.05$; fig. 2a).

eNOS and nNOS Expression in the Kidney Was Suppressed by BPS

Intraglomerular eNOS expressions in the kidneys of BPS-treated rats were apparently reduced compared to untreated rats (fig. 2b). In group C, the expression of nNOS was clearly upregulated in the macula densa. The expression was significantly suppressed in the kidneys of BPS-treated rats in a dose-dependent manner (fig. 2b).

Serum TG Concentration Was Improved by BPS

It was unexpected that there were significant differences in the levels of TG, but not in the levels of cholesterol, between BPS-treated and control groups in a dose-dependent manner. In fact, at 16 weeks after the beginning of administration, the TG of groups H, L, and C was 277 ± 43, 377 ± 65, and 578 ± 140 mg/dl, respectively (group H vs. C, $p < 0.01$; group L vs. C, $p < 0.05$; fig. 3a). Similarly, in LETO rats, significant differences in the levels of TG among BPS-treated and control groups were confirmed (data not shown). The results of HPLC analy-

Table 1. Chemistry values in OLETF and LETO rats

	Rat	Group	BPS-0w	BPS-4w	BPS-8w	BPS-12w	BPS-16w	p
Urinary protein, mg/day	OLETF	C	176.7 ± 36.9	215.3 ± 33.5	240.9 ± 34.1	250.6 ± 36.4	201.2 ± 34.6	
		L	168.2 ± 27.6	122.7 ± 23.3	152.3 ± 20.1	142.7 ± 26.2	176 ± 27.0	
		H	165.9 ± 24.1	101.7 ± 15.9	121.2 ± 16.0	162.9 ± 21.7	145.4 ± 22.8	<0.05
	LETO	C	7.9 ± 0.7	9.3 ± 0.5	9.6 ± 0.6	8.2 ± 0.7	10.3 ± 1.3	
		L	7.9 ± 0.8	5.2 ± 0.4	7.2 ± 0.8	11.3 ± 1.6	8.7 ± 0.8	
		H	8.2 ± 0.8	4.9 ± 0.5	7.1 ± 0.8	4.6 ± 0.7	8.6 ± 1.4	
BUN, mg/dl	OLETF	C	29.5 ± 0.8	33.9 ± 2.5	33.2 ± 3.2	37.5 ± 4.1	39.2 ± 5.3	
		L	27.3 ± 1.2	28.1 ± 2.2	28.3 ± 1.4	34.6 ± 1.9	31.3 ± 1.9	
		H	27.5 ± 1.2	25.3 ± 1.6	33.6 ± 1.4	31.3 ± 1.0	31.5 ± 0.9	<0.05
	LETO	C	29.7 ± 0.9	31.1 ± 0.8	30.3 ± 0.9	31.8 ± 0.7	30.2 ± 0.4	
		L	29.8 ± 0.9	34.4 ± 0.9	30.2 ± 0.7	34.7 ± 1.0	31.4 ± 1.2	
		H	30.9 ± 1.3	27.1 ± 1.0	34.4 ± 0.6	31.7 ± 1.2	28.8 ± 1.0	
Cr, mg/dl	OLETF	C	0.29 ± 0.01	0.29 ± 0.02	0.30 ± 0.03	0.33 ± 0.05	0.39 ± 0.07	
		L	0.27 ± 0.02	0.30 ± 0.03	0.34 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	
		H	0.26 ± 0.01	0.34 ± 0.01	0.28 ± 0.01	0.30 ± 0.01	0.36 ± 0.01	
	LETO	C	0.34 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.37 ± 0.01	0.37 ± 0.01	
		L	0.35 ± 0.01	0.48 ± 0.03	0.40 ± 0.01	0.43 ± 0.01	0.39 ± 0.01	
		H	0.34 ± 0.01	0.44 ± 0.02	0.38 ± 0.00	0.45 ± 0.07	0.44 ± 0.03	
TP, mg/dl	OLETF	C	6.9 ± 0.2	7.1 ± 0.2	7.8 ± 0.4	7.8 ± 0.4	7.6 ± 0.4	
		L	6.7 ± 0.2	7.1 ± 0.1	7.5 ± 0.3	7.3 ± 0.2	7.6 ± 0.2	
		H	6.7 ± 0.4	6.6 ± 0.2	7.3 ± 0.2	7.1 ± 0.1	7.3 ± 0.2	<0.05
	LETO	C	6.2 ± 0.2	6.8 ± 0.1	6.6 ± 0.2	6.6 ± 0.1	7.0 ± 0.1	
		L	6.3 ± 0.3	6.6 ± 0.1	6.0 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	
		H	6.2 ± 0.1	6.2 ± 0.1	5.8 ± 0.2	6.7 ± 0.3	6.5 ± 0.1	
Alb, g/dl	OLETF	C	3.9 ± 0.2	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	
		L	4.0 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	<0.05
		H	3.9 ± 0.2	4.2 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	<0.05
	LETO	C	4.2 ± 0.1	4.4 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.5 ± 0	
		L	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	
		H	4.2 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	4.3 ± 0.1	4.2 ± 0	
Glucose, mg/dl	OLETF	C	152.1 ± 23.0	188.3 ± 18.7	175.4 ± 40.6	178.3 ± 37.6	146.4 ± 23.6	
		L	142.7 ± 10.2	110.3 ± 6.4	124.4 ± 5.5	128.4 ± 17.5	127.1 ± 8.8	
		H	125.1 ± 12.7	119.9 ± 6.5	129.3 ± 10.6	135.7 ± 5.2	146.5 ± 6.6	
	LETO	C	108.4 ± 3.1	99.3 ± 5.5	99.3 ± 8.4	107.0 ± 3.0	111.4 ± 3.5	
		L	91.6 ± 10.6	99.6 ± 7.7	116.7 ± 6.1	79.9 ± 6.8	89.3 ± 3.21	
		H	93.0 ± 7.1	68.2 ± 12.5	116.0 ± 5.8	122.4 ± 3.8	98.4 ± 17.7	

Data are mean ± SEM.

sis of plasma lipoproteins showed that the chyromicron and very low density lipoprotein (VLDL) fractions in group H were apparently reduced compared with those in group C (fig. 3b), and there were significant differences in the levels of TG of each fraction among BPS-treated groups and the untreated group (fig. 3c).

Abnormal Lipid Depositions in Liver and Renal Glomeruli Were Attenuated by BPS

Lipid was exhibited as russet deposit with Sudan IV staining, and abundant deposits were detected in the glomerulus of the kidneys from group C. The degree of lipid deposition in the glomerulus of the kidneys from

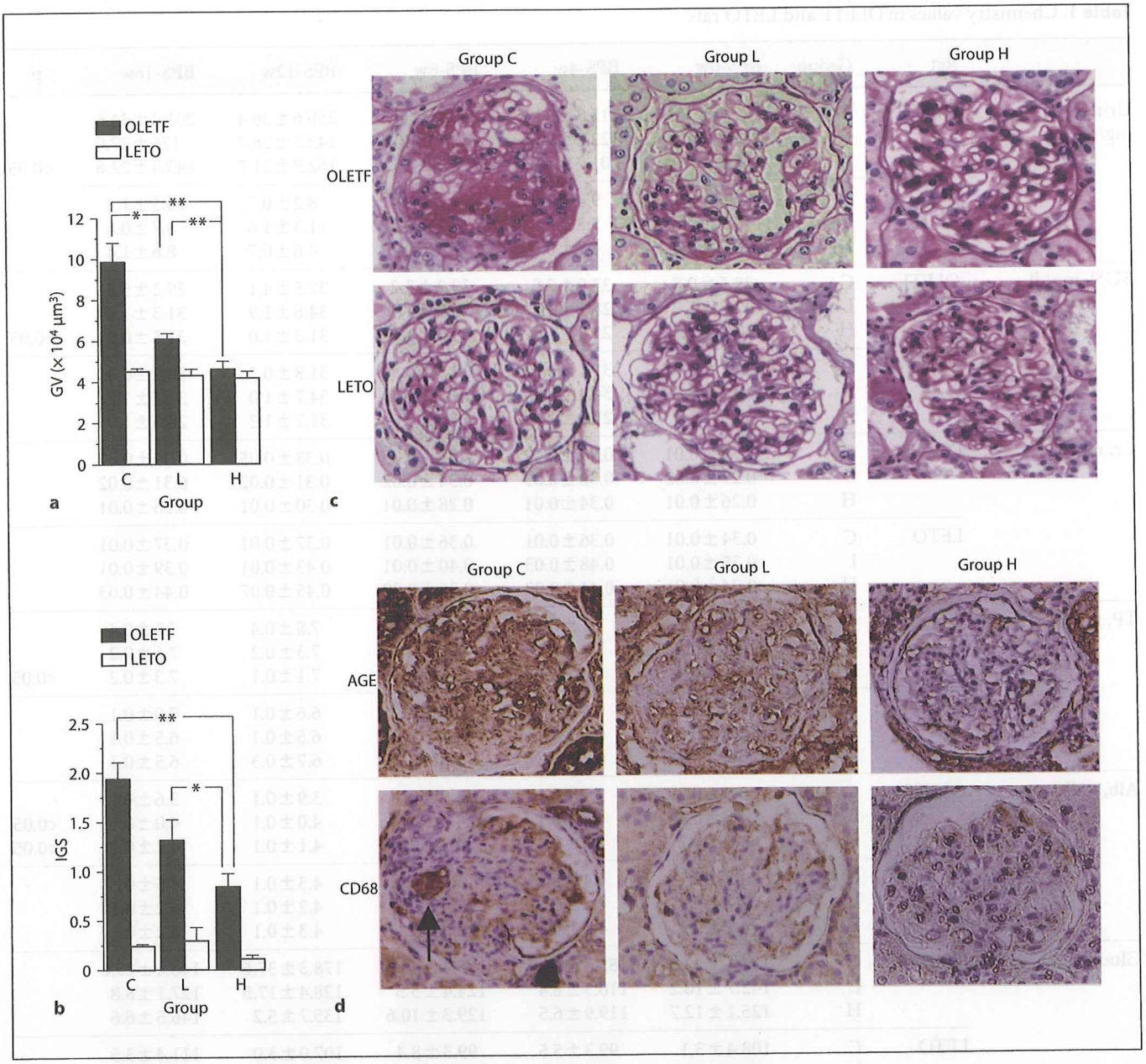

Fig. 1. BPS reduced IGS and GV, and improved histological findings of the kidneys in OLETF rats. **a** GV was significantly reduced in BPS treated OLETF rats compared to that in untreated OLETF rats. Evaluation conditions were equal to those for IGS determination. * $p < 0.01$; ** $p < 0.001$. **b** IGS was significantly reduced in BPS-treated OLETF rats compared with IGS in untreated OLETF rats. IGS was determined in kidneys from each group of rats sacrificed at 16 weeks after the beginning of BPS administration. * $p < 0.05$; ** $p < 0.01$. **c** Histological findings of glomeruli were different in OLETF rats among the three groups. Neither sclerotic change nor inflammatory cell infiltration was observed in the glomeruli of BPS-treated OLETF rats, similar to those in negative control, LETO rats. PAS. ×400. **d** The area of AGEs in the glomerulus and the numbers of intraglomerular macrophages of BPS-treated rats were reduced compared with untreated rats. The proportions of AGE area for glomerulus were presented by mean ± SE%. The number of intraglomerular macrophages was presented by mean number ± SE of anti-CD68 Ab-positive cells per glomerulus. The evaluations of AGE formation and macrophage influx in the glomeruli of the OLETF rats were performed by immunohistological method using anti-AGE Ab and anti-CD68 Ab. ×400. Arrow indicates intraglomerular foam cell.

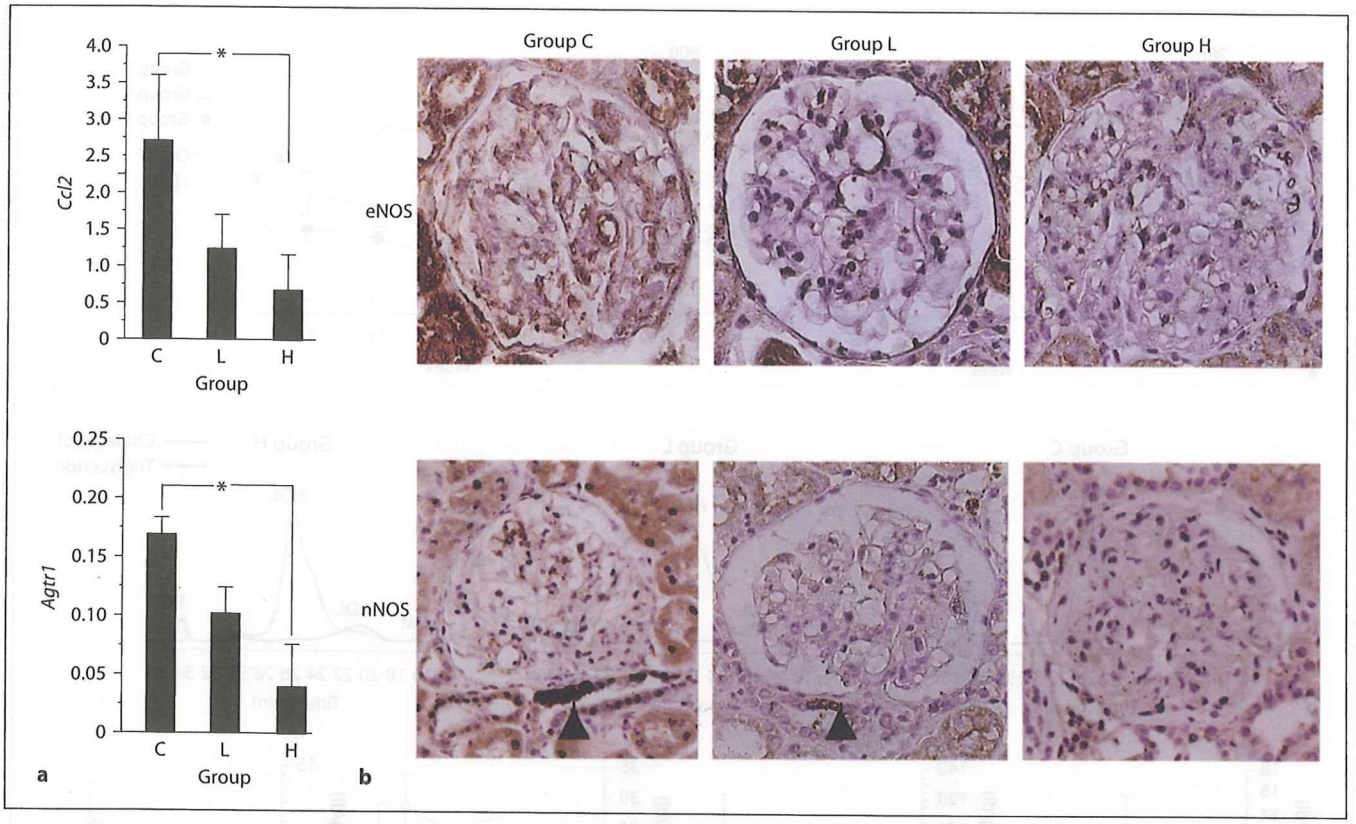

Fig. 2. BPS reduced the mRNA expression of *Ccl2 and Agtr1*, and inhibited the expression of eNOS and nNOS of the kidneys in OLETF rats. **a** Real-time RT-PCR quantifications of *Ccl2* and *Agtr1* mRNA expressions in the kidneys of rats in groups C, L, and H (n = 7 each). The relative abundance of mRNAs normalized by β-actin is shown. * $p < 0.05$. **b** Representative findings of eNOS and nNOS expressions in the kidneys of rats in groups C, L, and H. Arrows indicate upregulation of nNOS in the macula densa.

BPS-treated groups was attenuated in a dose-dependent manner. The deposition was hardly seen in the glomerulus of the kidneys from group H. Similarly, apparent lipid depositions around the central vein were revealed in liver specimens of group C, but not group H (fig. 4).

Discussion

OLETF rat was established as an animal model of human type 2 diabetes that exhibits obesity, hyperinsulinemia, hypertriglycemia, and hyperglycemia [17]. The pathological changes in the kidneys were described as follows: mesangial cell proliferation at 25 weeks of age, mesangial expansion accompanied by the accumulation of extracellular matrix and thickening of glomerular capillary walls after 45 weeks, and fully developed diabetic glomerulopathy accompanied by nodular sclerosis at 65 weeks. In LETO rats, there were no obvious findings except minor alterations due to aging in the glomeruli even at 65 weeks [18].

In this study, we demonstrated that BPS attenuated the severity of diabetic nephropathy in OLETF rats. There were significant differences in urinary protein and serum levels of BUN, Alb and TP between the BPS-treated group H and the untreated (group C) OLETF rats (table 1). IGS and GV among the 3 groups also showed significant differences in a dose-dependent manner (fig. 1a, b). Light microscopic examination showed the kidneys of BPS-treated rats were protected against glomerulosclerosis (fig. 1b). Wang et al. [6] reported that the value of albuminuria excretion in STZ-induced diabetes rats was reduced by administration of BPS. They demonstrated that BPS corrected glomerular hyperfiltration and decreased albuminuria of early diabetic nephropathy. It was also demonstrated that BPS decreased the expression of endo-

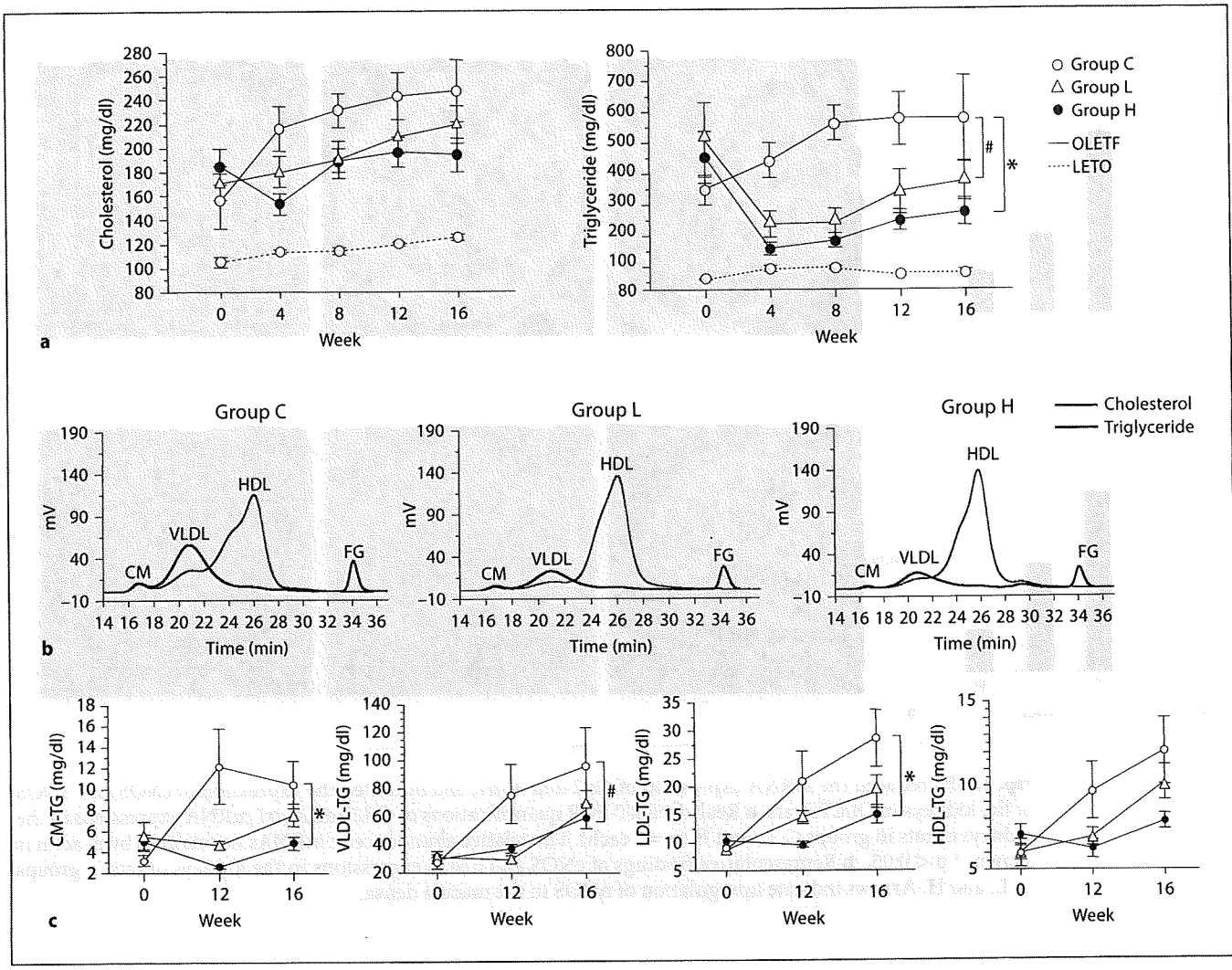

Fig. 3. BPS altered plasma lipoprotein levels in OLETF and LETO rats. **a** Serum levels of TC and TGs were determined every 4 weeks. Data shown are mean ± SE of each group. **b** Representative results of HPLC analyses of plasma lipoproteins in group C, L and H OLETF rats. CM = Chyromicron; VLDL = very low density lipoprotein; LDL = low density lipoprotein; HDL = high density lipoprotein; FG = free glycerol. **c** TG levels in lipoprotein subclasses (CM, VLDL, LDL and HDL) were determined. Data shown are mean ± SE in each group. Significant differences are indicated as follows: * $p < 0.01$; # $p < 0.05$.

thelial cell nitric oxide synthetase in afferent arterioles and improved glomerular hyperfiltration in STZ-induced diabetic rats [8]. In the current study, the efficacy of BPS on diabetic nephropathy of OLETF rats was confirmed. The sclerotic changes due to extracellular matrix accumulation or capillary wall thickening in the glomeruli of BPS-treated rats were significantly suppressed (fig. 1b) and GVs were clearly reduced. These histological alterations may result from the improvement of glomerular hyperfiltration by BPS.

There is evidence indicating that formation and accumulation of AGE in diabetic nephropathy mediates progressive alteration in renal architecture and loss of renal function [19–21]. Hyperglycemia induces overproduction of superoxide by the mitochondrial electron transport chain, and this superoxide partially inhibits the glycolytic enzyme glyceraldehyde phosphate dehydrogenase [22, 23]. Thereby, upstream metabolites of glycolysis are diverted into glucose-driven signaling pathways of glucose overuse [23, 24]. Especially the accumulation of glyceral-

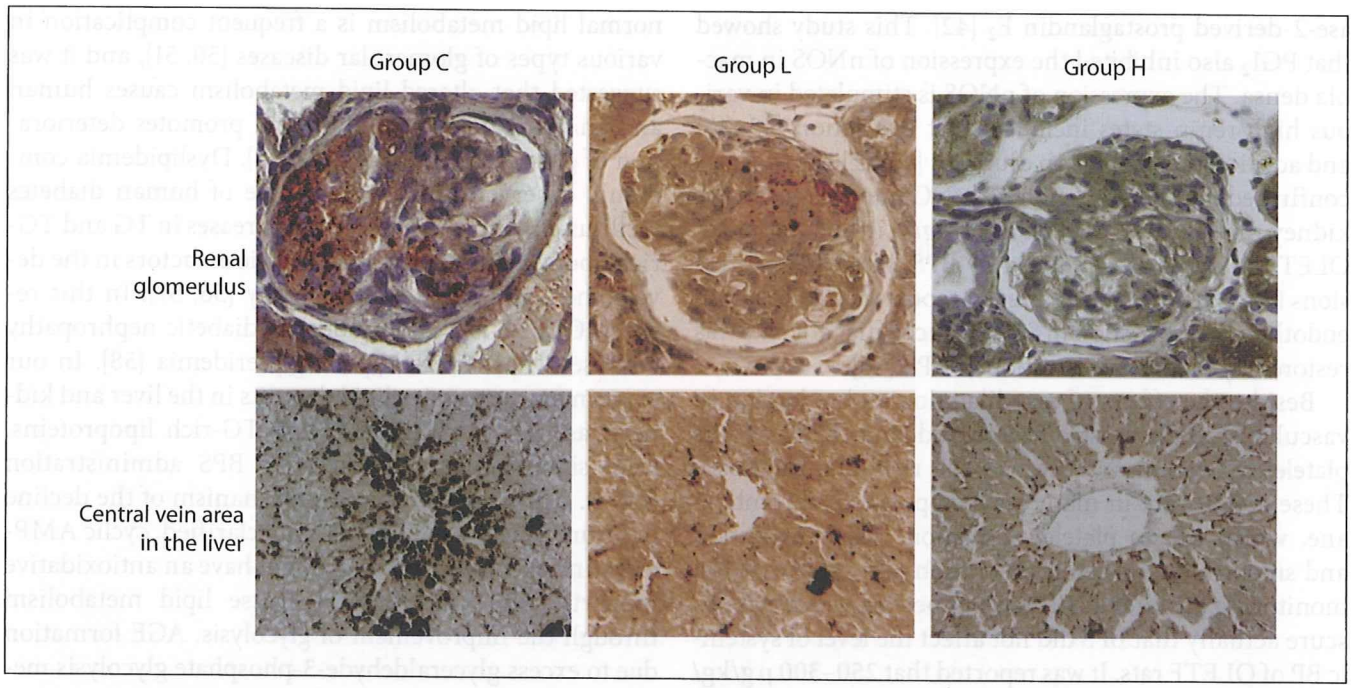

Fig. 4. BPS attenuated abnormal lipid deposition in the kidneys and liver. Representative results of specimens stained with Sudan IV. Apparent russet deposits were detected within the renal glomerulus and central vein area in the liver from group C OLETF rats.

dehyde-3-phosphate accelerates intracellular AGE formation [25], which promotes the secretion of MCP-1 in mesangial cells [26]. Monocyte/macrophage infiltration has been observed in the glomerulus in human and experimental diabetes and is involved in diabetic nephropathy [27–29]. AGE-induced vascular wall cell apoptosis could predispose the neighboring endothelial cells to thrombogenesis by impairing PGI_2 production and thus be implicated in the development and progression of diabetic microangiopathies [30]. In the current study, immunohistological findings revealed that the formation of AGEs in the glomerulus of BPS-treated rats was apparently reduced in a dose-dependent manner (fig. 1d). Intraglomerular macrophage influx and subsequent foamy changes are found in some glomeruli of untreated OLETF rats. In contrast, the glomeruli in BPS-treated rats were protected against macrophage influx, and did not contain any foamy cells (fig. 1d). Monocytes are recruited and activated by MCP-1 at the inflammatory sites [31], and BPS could suppress MCP-1 production by reduction of AGE formation. In fact, a significant reduction in the expression of mRNA for MCP-1 in the kidneys of the BPS-treated rats (fig. 2a) is further evidence that BPS inhibits the MCP-1 signaling pathway in the kidneys of OLETF rats.

Among many enzymatic systems that are capable of producing superoxide, the nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase and the uncoupled eNOS are the main sources of superoxide in the vascular wall in diabetic patients [32]. It was also reported that NADPH oxidase and uncoupled eNOS were major sources of glomerular superoxide in rat diabetic kidney [33]. It was indicated that early diabetic proteinuric nephropathy was associated with increased expression of p47phox component of the reduced form of NADPH oxidase and eNOS with increased H_2O_2 formation in the kidney, and treatment with an ACE inhibitor or ARB reversed these changes with amelioration of proteinuria [34]. Many studies have shown increased eNOS expression [35–37] and eNOS activity [38, 39], especially in kidneys from early stages of diabetes. In this study, histopathological examination showed that eNOS expression in the glomerulus of untreated OLETF rats was increased and that BPS treatment suppressed this expression. Furthermore, nNOS expression in the macula densa was also suppressed by BPS treatment (fig. 2d). It is well established that cyclooxygenase-2 and nNOS are coexpressed in macula densa cells [40, 41], and it has been described that nNOS expression in the macula densa was inhibited by cyclooxygen-

ase-2-derived prostaglandin E_2 [42]. This study showed that PGI_2 also inhibited the expression of nNOS in macula densa. The expression of nNOS is stimulated in various high-renin states including salt restriction [43–45] and administration of loop diuretics [43]. These findings confirmed the strong angiotensin-NO interactions in the kidney. In fact, the expression of *AgtrI* in the kidney of OLETF rats was also reduced by BPS, like NOS expressions (fig. 2b). It has been already reported that impaired endothelial dysfunction in STZ-induced diabetic rats was restored by oral administration of BPS [46].

Besides the effects of vasodilatation and reduction in vascular smooth muscle cell remodeling, BPS inhibits platelet activation and reduces the risk of thrombosis. These actions are in many ways opposite to thromboxane, which causes platelet activation, vasoconstriction and smooth muscle cell proliferation [47]. In this study, monitoring of systemic BP was not performed. It was obscure actually that BPS did not affect the level of systemic BP of OLETF rats. It was reported that 250–300 μg/kg/day of BPS does not affect systemic BP in rats [48]. While we treated 50-week-old OLETF rats with 400 μg/kg per day (group H) and 200 μg/kg per day (group L), it is sufficiently possible that the levels of systemic BP in OLETF rats would have been lowered by BPS treatment. Although it was described that temporary angiotensin II blockade did not affect glucose metabolism or the development of hypertension in OLETF rats but significantly suppressed proteinuria and ameliorated glomerular injury [49], decrease in systemic BP would be a significant factor for the improvement of diabetic nephropathy in OLETF rats.

Intriguingly, BPS reduced the level of serum TG in not only OLETF, but also LETO rats (fig. 3a and data not shown). Although it is ambiguous whether or not lipoprotein profiles in rats are similar to those of humans, the reduction in the VLDL fraction was obvious (fig. 3b). Abnormal lipid metabolism is a frequent complication in various types of glomerular diseases [50, 51], and it was suggested that altered lipid metabolism causes human and animal glomerular injury and promotes deterioration of glomerular function [52–54]. Dyslipidemia commonly observed during the course of human diabetes mellitus [55] is characterized by increases in TG and TG-rich lipoproteins, which are deleterious factors in the development of diabetic nephropathy [56, 57]. In this respect, OLETF is a good model of diabetic nephropathy because it has severe hypertriglyceridemia [58]. In our experiments, excessive lipid deposits in the liver and kidneys, as well as plasma TG and TG-rich lipoproteins, were significantly reduced after BPS administration (fig. 4). Although the detailed mechanism of the decline in serum TG levels by BPS was not clarified, cyclic AMP-elevating agents such as BPS might have an antioxidative property [59], and might increase lipid metabolism through the improvement of glycolysis. AGE formation due to excess glyceraldehyde-3-phosphate glycolysis metabolites might be suppressed by the same mechanism.

In summary, our findings provide in vivo evidence that BPS attenuates the severity of diabetic nephropathy in OLETF rats. BPS significantly reduced IGS and GV. In this study, it is also apparent that BPS reduces the serum level of TG in both OLETF and LETO rats. These findings indicate that BPS has a pleiotropic therapeutic effect on the diabetic nephropathy of OLETF rats, which suggests a potential application of this drug in the treatment of human diabetic nephropathy.

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