concentration. OA/BSA caused a slight increase in apoptosis, but the difference was not statistically significant. The result indicates that, irrespective of whether BSA alone can induce apoptosis, FFAs bound with BSA can exert additional effects to induce apoptosis of human PTCs, and that the amount of FFAs included in the normal rBSA preparation was sufficient for the effect. This may explain the origin of oval fat bodies in the urine, which are likely to be cell debris after fatty degeneration.[26] The observed apoptosis did not occur because of lipopolysaccharide contaminating BSAs,[27] because rBSA with little endotoxin contamination (<0.1 ng/mg, A9306; Sigma) caused apoptosis in the present experiment.

The effect of FFAs was also examined using human HK-2 cells, which have been used as a model of PTCs in many studies.[28–30] An increase in the apoptotic ratio (Figure 2B) and a decrease in the surviving cell ratio (Figure 2A) were observed by FFA treatments. The relatively low apoptotic ratio in HK-2 cells was probably because they are transformed cells. Nonetheless, the result was essentially the same as that obtained in human PTCs. We further examined the dose effect of rBSA on apoptotic induction. As shown in Figure 2C, the apoptotic ratio increased in a dose-dependent manner, and at any concentration the effect was larger than dBSA at the same concentration.

We also examined the effect of FFAs that was applied only to the apical surface of the polarized PTC using LLC-PK1 cultured on a Transwell filter support. This experiment was done because PTC *in vivo* is the simple epithelium with a tight intercellular barrier and FFA-bound albumin bathes the apical surface alone in nephrosis. Formation of an impermeable monolayer was confirmed both by the *trans*-epithelial resistance measurement and by immunofluorescence microscopy of ZO-1 (data not shown). When various BSA preparations were added to the apical chamber, rBSA and LA/dBSA, but not OA/dBSA, increased the apoptotic ratio than dBSA in both TUNEL and FITC-VAD-FMK assays (Supplementary Figure S1, see *http://ajp.amjpathol.org*). This result showed that exposure of the apical surface of polarized epithelial cells to FFAs is sufficient to induce apoptosis.

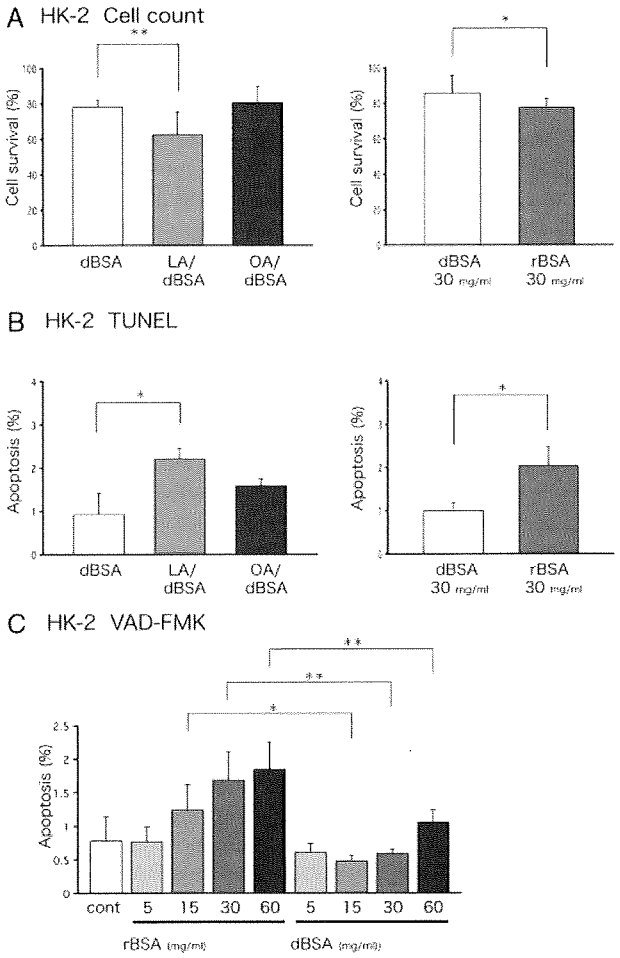

Figure 2. Apoptosis in HK-2 cells. **A:** HK-2 cells, preincubated for 24 hours in serum-free medium, were treated with dBSA (4.4 mg/ml), LA/dBSA, OA/dBSA (FFAs, 400 μmol/L; BSA, 4.4 mg/ml), dBSA (30 mg/ml), or rBSA (30 mg/ml; FFA concentration, 185 μmol/L) for another 24 hours. Cell survival was analyzed with the Cell Counting Kit 8. The number of surviving cells are shown as a ratio to that of control cells kept in 10% FCS. The results of nine samples obtained in three independent experiments were averaged (mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$). **B:** HK-2 cells treated in the same manner as described in **A** were analyzed by TUNEL assay. The number of positive cells was counted in eight random areas in three independent experiments and averaged (mean ± SD, $^{*}P < 0.05$). **C:** HK-2 cells were preincubated for 24 hours with 0.5% lipoprotein-deficient serum (LPDS), treated with dBSA or rBSA (5, 15, 30, 60 mg/ml) for another 24 hours, stained by FITC-VAD-FMK, and analyzed by flow cytometry. Control cells were kept in 0.5% LPDS without any further additions. Results obtained in three independent experiments were averaged (mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$).

FFA-Bound Albumin Increases LDs, ADRP, and TIP47 in Human PTCs

In many cell types including PTCs,[16] FFAs were shown to increase the total volume of LDs and expression of LD-associated proteins. We examined whether similar increases occur in human PTCs using the above treatments that induced apoptosis. In human PTCs treated with dBSA alone, either at 4.4 mg/ml or 30 mg/ml, LDs were hardly visible by BODIPY493/503 staining, and labeling for ADRP or TIP47 was negligible (Figure 3A). After treatment with LA/dBSA, OA/BSA, or rBSA, LDs were clearly observed and were positively labeled for ADRP and TIP47. Quantification of the labeling intensity in randomly taken fluorescence micrographs showed that both ADRP and TIP47 labeling in LDs increased after FFA-bound albumin treatment (Figure 3, B and C). A very similar result was obtained in HK-2 cells and after the DHA/dBSA treatment (data not shown). TIP47 can exist stably both as soluble and LD-bound forms, and is rapidly recruited from the cytosol to LDs when FFAs are administered.[31,32] On the other hand, ADRP exists in LDs constitutively and is a more reliable marker for estimating the amount of LDs. Consistent with this, Western blotting showed that the total expression of ADRP was greater in cells treated with rBSA than in those treated with dBSA, whereas the expression of TIP47 was not significantly different in the two samples (Figure 3D). An increase of ADRP in LA/dBSA- and OA/dBSA-treated cells was also confirmed by Western blotting (data not shown).

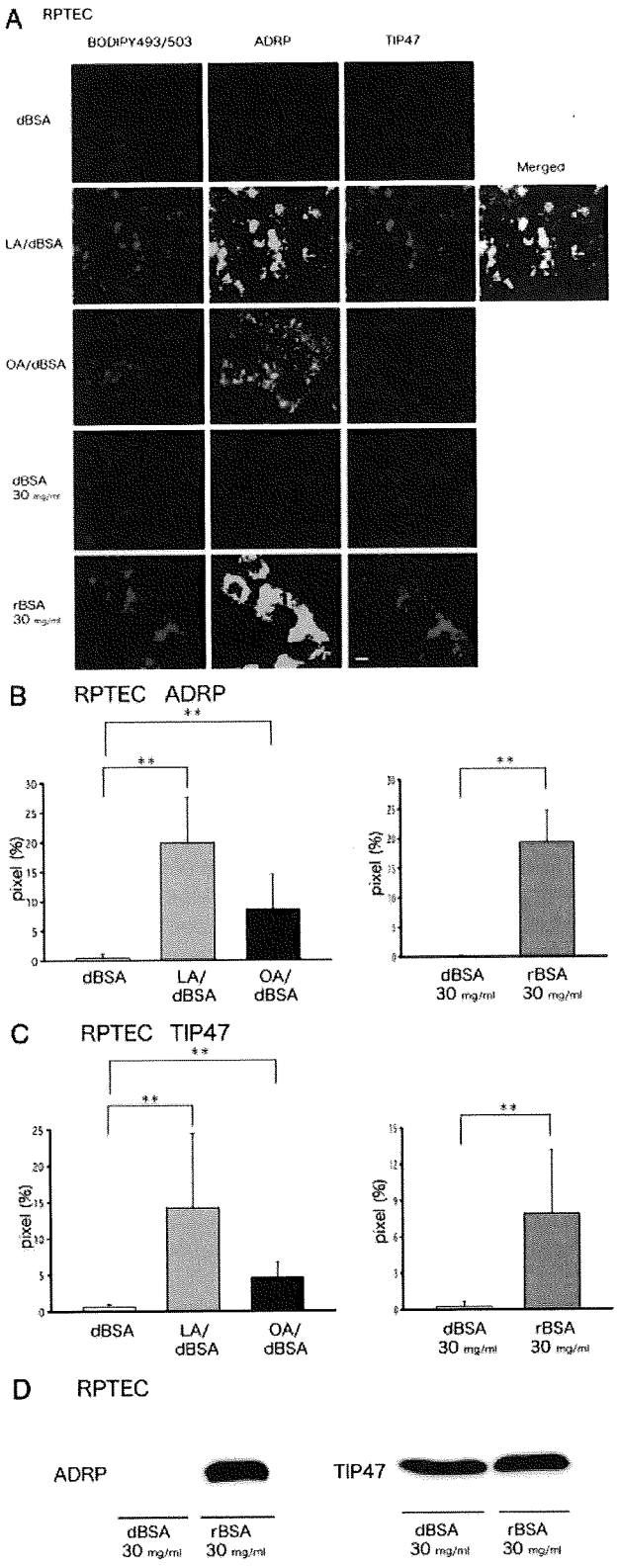

Figure 3. LDs and LD-associated proteins in human PTCs (RPTECs) increase significantly on treatment with FFAs bound to BSA. **A:** Human PTCs were treated with dBSA (4.4 mg/ml), LA/dBSA, OA/dBSA (FFAs, 400 μmol/L; BSA, 4.4 mg/ml), dBSA (30 mg/ml), or rBSA (30 mg/ml) for 24 hours as described in Figure 1. LDs were stained by BODIPY493/503 (green), whereas ADRP (blue) and TIP47 (red) were labeled with antibodies. A merged picture showed clearly that ADRP and TIP47 co-localize in the same LDs. Treatment with LA/dBSA, OA/dBSA, or rBSA significantly increased the labeling intensity of LDs, ADRP, and TIP47. **B** and **C:** The labeling intensity of ADRP (**B**) and TIP47 (**C**) by immunofluorescence microscopy was quantified (mean ± SD). Results obtained in three independent experiments were averaged. Eight areas were randomly chosen and photographed at the same setting. The ordinate is the percentage of pixels that shows the labeling more intense than a threshold (mean ± SD, $^{**}P < 0.01$) **D:** Western blotting of ADRP and TIP47 using the total cell lysate. Treatment with rBSA (30 mg/ml) drastically increased the expression of ADRP, whereas the influence on TIP47 expression was minimal. Equal amounts (30 μg) of protein were loaded in each lane. Scale bar = 5 μm.

Antioxidants Suppress FFA-Induced Apoptosis to Various Extents

Polyunsaturated FFAs (PUFAs) are substrates of iron-dependent peroxidation reactions that give rise to lipid peroxides, or reactive lipid oxygen species, and increase oxidant stress.[33,34] To explore whether lipid peroxidation is involved in FFA-induced apoptosis, we examined the effect of antioxidants: vitamin E (120 μmol/L) and desferrioxamine (100 μmol/L), a specific chelator for iron that inhibits iron-dependent lipid peroxidation,[35] were used. After preincubation with vitamin E or desferrioxamine for 1 hour, human PTCs were treated with LA/dBSA or rBSA for another 24 hours in the continued presence of antioxidant. Apoptosis induced by LA/dBSA was suppressed by incubation with either vitamin E or desferrioxamine (Figure 4A), but was still significantly more than that caused by dBSA alone (Figure 4B). In contrast, apoptosis induced by rBSA was not affected by the presence of antioxidants (Figure 4C). Similar results were obtained in HK-2 cells by flow cytometry after FITC-VAD-FMK staining (data not shown). Interestingly, apoptosis caused by DHA/dBSA in HK-2 cells was completely suppressed by antioxidants (Supplementary Figure S2, see *http://ajp.amjpathol.org*). Consistent with these results, the TBARS assay showed that DHA/dBSA is the strongest oxidative stress, whereas LA/dBSA is significantly weaker than DHA/dBSA, and rBSA was comparable to dBSA (Supplementary Figure S2C, see *http://ajp.amjpathol.org*). The results corroborate that lipid peroxidation is the major cause of apoptotic induction by polyunsaturated (24:6) DHA/dBSA, whereas it is only partially involved in the induction by LA/dBSA containing two unsaturated bonds (18:2) and is not related to the induction by rBSA primarily consisting of saturated fatty acids.

Knockdown of ADRP and TIP47 Augments FFA-Induced Apoptosis

The experiments described above show that treatment with FFA-bound albumin increases LD formation and apoptosis in PTCs. By storing excess FFAs as lipid esters, LDs are thought to protect cells against the toxicity of FFAs.[36] This led to speculation that manipulation of LD formation may influence the outcome of FFA loading. To test this hypothesis in PTCs, we knocked down expression of ADRP and TIP47 by RNAi and examined the effect on FFA-induced apoptosis.

The RNAi procedure using electroporation significantly decreased the expression of both ADRP and TIP47 in human primary PTCs, although the effect was consistently more pronounced for TIP47 than for ADRP (Figure

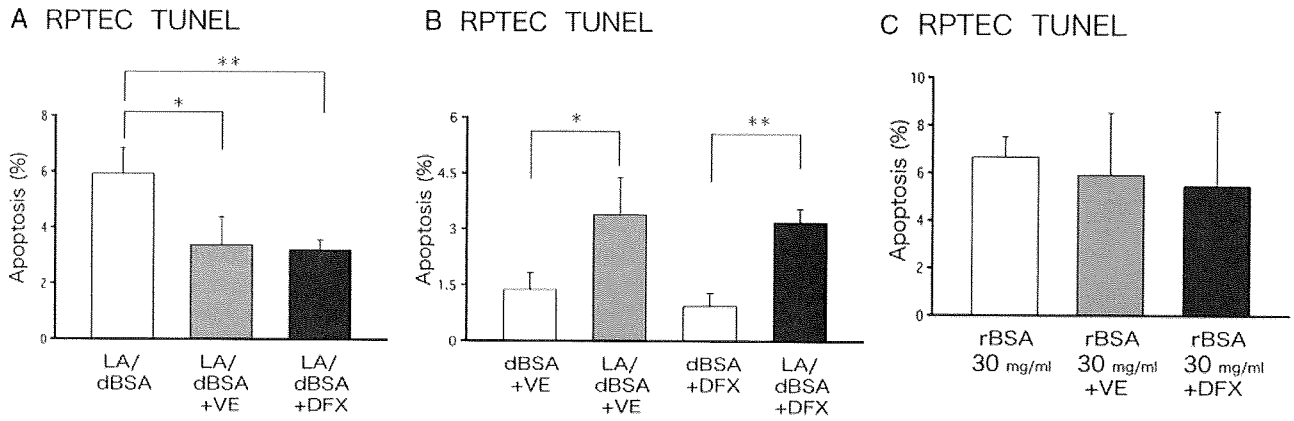

Figure 4. Increased FFA-induced apoptosis persists in the presence of antioxidants. Human PTCs were preincubated with 120 μmol/L vitamin E (VE) or 100 μmol/L desferrioxamine (DFX) for 1 hour, and were then incubated with LA/dBSA (LA, 400 μmol/L; BSA, 4.4 mg/ml) or rBSA (30 mg/ml) for 24 hours. Apoptosis was measured by the TUNEL assay. **A:** Vitamin E and desferrioxamine significantly decreased apoptosis induced by LA/dBSA. **B:** Even in the presence of vitamin E or desferrioxamine, LA/dBSA caused a higher ratio of apoptotic cells than dBSA alone. **C:** Apoptosis induced by rBSA was not significantly reduced by vitamin E or desferrioxamine. Results obtained in three independent experiments were averaged (mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$).

5A). Two days after RNAi, the cells were treated with LA/dBSA or rBSA, and the apoptotic ratio was examined 24 hours later by the TUNEL assay. The apoptotic ratio was compared between cells transfected with random control siRNA and those treated with siRNAs specific for ADRP or TIP47. Knockdown of either ADRP or TIP47 significantly increased the ratio of apoptotic cells that were induced by both LA/dBSA (Figure 5B) and rBSA (Figure 5C). A similar result was obtained by quantifying the nuclear condensation after the 4,6-diamidino-2-phenylindole staining (Supplementary Figure S3, see *http://ajp.amjpathol.org*). A basal level of apoptosis observed in the presence of dBSA was not changed by the RNAi procedure. A similar result was obtained in HK-2 cells that were subjected to RNAi and examined by flow cytometry after FITC-VAD-FMK staining (data not shown). These results suggest that endogenous ADRP and TIP47 may protect cells from apoptosis by augmenting storage of FFAs as lipid esters in LDs.

Discussion

In the present study, we first showed that dBSA bound with LA or rBSA induced more apoptosis than dBSA at the same concentration using three different cell preparations: human primary PTCs and two immortalized cell lines derived from PTCs, HK-2 and LLC-PK1. Second, we demonstrated that knockdown of either ADRP or TIP47 aggravated FFA-induced apoptosis, suggesting a protective role of those LD-associated proteins in PTCs.

Concerning the first point, the result of LLC-PK1 cells is important because they retain the apico-basolateral polarization when cultured on semipermeable supports and form tight junctions to demarcate the apical and basal fluid compartments. During nephrosis, a drastic increase in protein concentration in the renal tubular fluid causes various changes in the epithelial cells, whereas the basolateral environment should remain primarily unaffected at least during the initial stage. This condition could be produced *in vitro* by adding BSA to only the apical compartment of polarized LLC-PK1 cells. In the present experiment, apoptosis was similarly induced in nonpolarized human PTCs and HK-2 cells that were exposed to FFA-bound BSAs on all surfaces. But in other experimental conditions, loss of polarization could cause changes in cellular reactions. Conflicting results have been reported concerning the effect of BSA on cultured renal tubular cells,[7,9–11] and we speculate that some of these discrepancies could be related to lack of cellular polarization in the experimental systems.

FFAs can induce apoptosis through different pathways, and the pro-apoptotic potency of each FFA may vary depending on the cell types.[36] In the present study, rBSA and LA/dBSA caused more apoptosis than OA/dBSA in PTCs. Because the induction of apoptosis by DHA/dBSA in HK-2 cells was completely suppressed by either the antioxidant vitamin E or the iron-chelator desferrioxamine, oxidative stress appears to be the major cause of apoptosis in this case, as previously suggested.[37] However, apoptosis caused by LA/dBSA was only partially suppressed by these reagents, and apoptosis caused by rBSA, primarily bound with saturated FFAs, was not affected at all. This result suggests that oxidative stress is a major effect that PUFAs exert on cells, but that FFAs with less unsaturation induce apoptosis by other mechanisms. That is, if human serum albumin is primarily bound with saturated FFAs as rBSA, antioxidants would not be effective to block apoptosis in PTCs of the nephrotic kidney.

In many cases including the present study, OA has been shown to be less toxic than saturated FFAs or PUFAs.[38] It may be partly because monounsaturated OA may not generate strong oxidative stress and is not a precursor for ceramide synthesis.[38] Additionally, OA may counterbalance its toxicity by promoting LD formation, thereby reducing the FFA concentration.[36] This supposition can be extended to other FFAs, and we speculated that the proapoptotic potency of each FFA can be determined by the balance between its toxicity and ability to induce LD formation. From this viewpoint, we hypothesized that reduction of the LD-associated proteins, ADRP and TIP47, would compromise the capacity of cells to

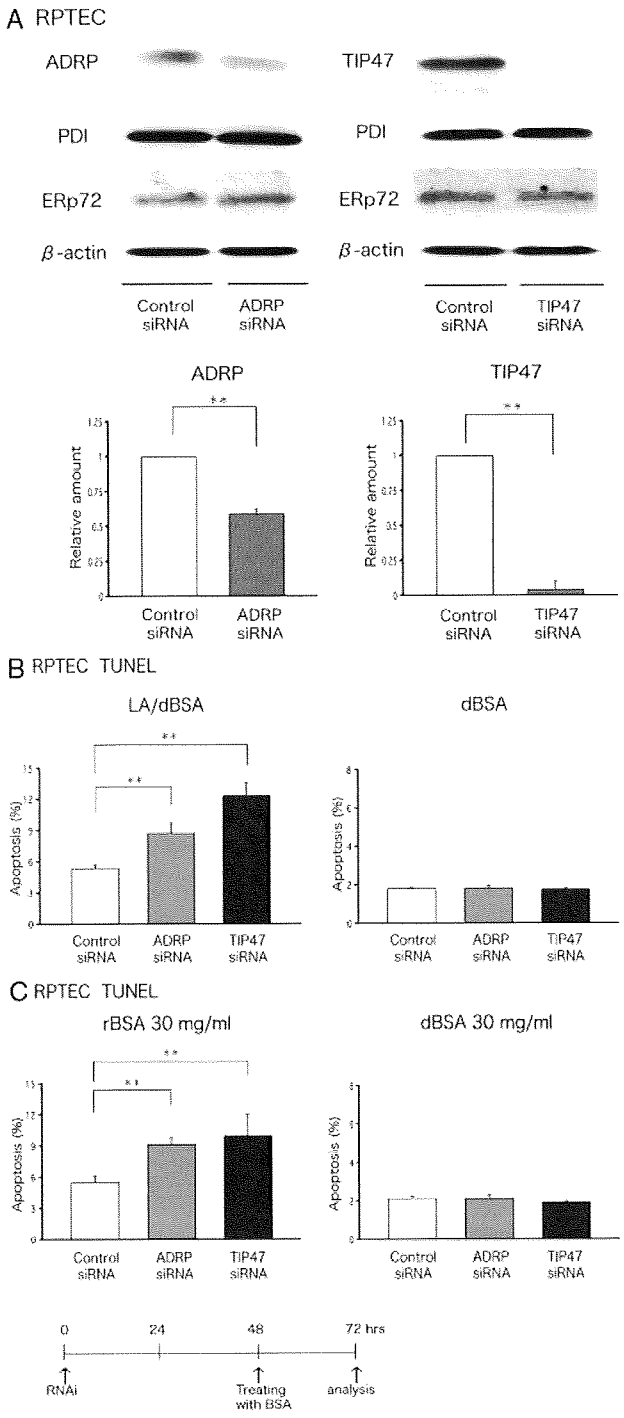

Figure 5. Knockdown of ADRP or TIP47 augments FFA-induced apoptosis in human PTCs (RPTECs). **A:** Western blotting showed that expression of ADRP and TIP47 in human PTCs was reduced significantly by RNAi. Equal amounts (30 μg) of total cell lysate were electrophoresed and probed with antibodies to ADRP, TIP47, PDI, ERp72, and β-actin. The RNAi of ADRP and TIP47 reduced expression of respective protein significantly, but did not affect other proteins. Results obtained from three independent experiments were averaged and shown in the bar graph (mean ± SD). **B** and **C:** Human PTCs were transfected with either random, ADRP, or TIP47 siRNA, and then challenged with LA/dBSA, rBSA, or dBSA. Apoptosis was measured by the TUNEL assay. The result is shown as the average of four independent experiments (mean ± SD, $^{**}P < 0.01$). **B:** Knockdown of either ADRP or TIP47 increases apoptosis induced by LA/dBSA (LA, 400 μmol/L; BSA, 4.4 mg/ml), whereas it does not influence the basal level of apoptosis observed in the presence of dBSA (4.4 mg/ml). **C:** The knockdown also increased apoptosis by 30 mg/ml of rBSA, but it did not affect the apoptotic ratio in cells treated by the same concentration of dBSA.

store lipid esters and increase apoptosis on administration of FFAs. As expected, when either ADRP or TIP47 was down-regulated by RNAi, apoptosis induced by LA/dBSA or rBSA was significantly enhanced. The effect on apoptosis was more consistently observed with TIP47 RNAi than with ADRP RNAi. This difference could result from potential critical roles played by TIP47 in sequestering FFAs to LDs, but it may simply be explained by the fact that knockdown of TIP47 was more efficient than ADRP.

ADRP has been presumed to be involved in the generation of LDs.[21,39,40] Consistent with this supposition, ADRP-null mice show a drastic reduction of LDs and lipid esters in the liver.[41] Perilipin, a LD-associated protein expressed in adipocytes and steroidogenic cells, prevents cytosolic hormone-sensitive lipases from acting on lipid esters in LDs.[42,43] ADRP may also have a similar protective role against lipases,[44] and may also reduce the cytotoxic effect of FFAs by functioning in LDs in an undefined manner. On the other hand, the function of TIP47 with regards to LDs is unclear,[18,31,45] whereas its involvement in recycling of the mannose-6-phosphate receptor from endosomes to the *trans*-Golgi network has been reported.[46] TIP47 shows significant similarity to ADRP both in amino acid sequence and three-dimensional structure[45,47] and is likely to have a related function in some aspect of lipid metabolism. Even though the molecular mechanism is not clear, we speculate that reduction of ADRP and TIP47 compromised the ability of cells to sequester FFAs as lipid esters and thus led to an increase in FFA-induced apoptosis in PTCs (Figure 6).

We expected that cDNA transfection of ADRP or TIP47 would provide a protective effect against FFA-induced apoptosis, but it did not cause a significant change in HK-2 cells (data not shown). We speculate that this negative result was primarily attributable to the low level of protein overexpression after cDNA transfection (data not shown). Expression of ADRP is posttranslationally regulated through polyubiquitylation and proteasomal degradation.[48,49] Without binding to LDs, they are likely to be short-lived, and thus an introduction of cDNA did not lead to a significant increase of protein. The regulatory mechanism of TIP47 protein expression is not known, but the lack of a significant increase in expression after cDNA transfection suggests a similar mechanism. Additionally, different LD-related proteins may cooperate with each other, such that they may need to be simultaneously overexpressed to be truly functional. That is, if expression of ADRP, TIP47, and some other proteins can be physiologically increased, they may give rise to an increase in protective function. In this context, it is noteworthy that PPAR ligands inhibited progression of diabetic nephropathy[50–52] and experimental glomerulonephritis.[9,53] This phenotype may be the result of an increase of not only ADRP but also other LD-related proteins.[15,54,55]

The present study shows that FFAs bound to BSA induce apoptosis in PTCs, and that reduction of ADRP or TIP47 further increases the ratio of apoptotic cells. This result does not exclude the possibility that high concentrations of urinary proteins alone can affect renal tubular cells in the nephrotic kidney. Most importantly, this study

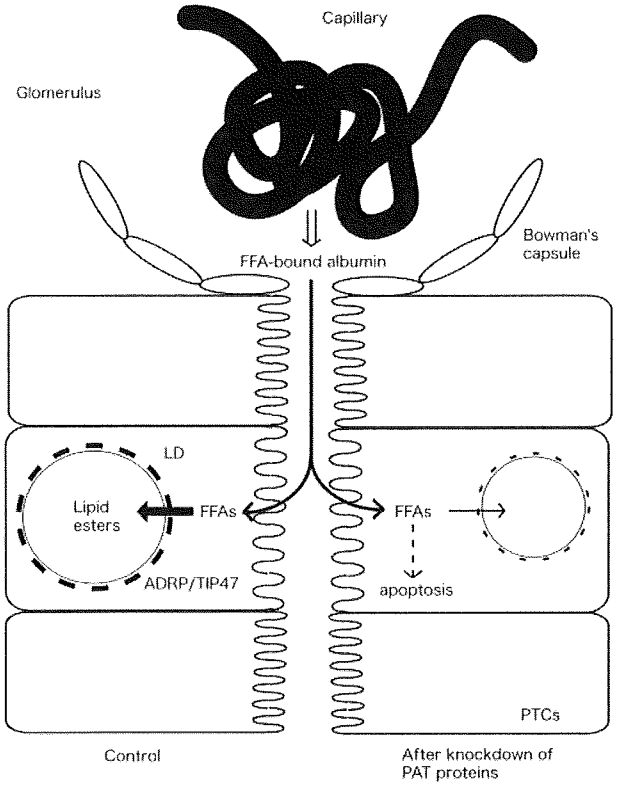

Figure 6. Based on the results of the present study, we hypothesize that LD and LD-associated proteins function in PTCs of the nephrotic kidney as described in the figure. Albumin that reached the tubular lumen presents FFAs to PTCs in an unregulated manner. FFAs may induce apoptosis in PTCs, but LDs can sequester FFAs as lipid esters and thereby protect cells from their toxicity. When the LD-associated proteins were reduced, FFA-sequestering ability of LDs may be compromised, and the resultant increase of FFAs in the cytosol may induce an increase in apoptosis.

demonstrates for the first time that LD-associated proteins play some protective role against FFA-induced cytotoxicity in PTCs. Furthermore, it reveals that the cellular toxicity of FFAs is heterogeneous and not linearly correlated with the number of double bonds. The result suggests that the prognosis for nephrosis may vary depending on the expression level of LD-associated proteins and composition of FFAs bound to serum proteins. It also suggests that LD-associated proteins and their regulatory mechanisms may be used as targets of therapeutic intervention for nephrotic diseases. We hope that the role of LD-associated proteins in PTCs will be confirmed in animal experiments and translated to clinical applications in the near future.

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医療／薬品

腎臓・膀胱 微小循環と再生

医学系研究科　機能構築医学専攻
病態外科学　泌尿器科

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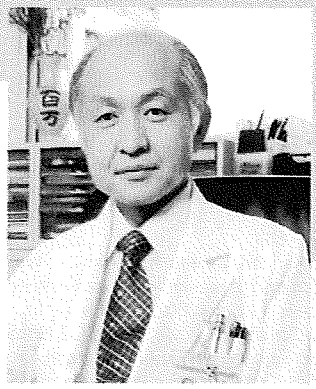

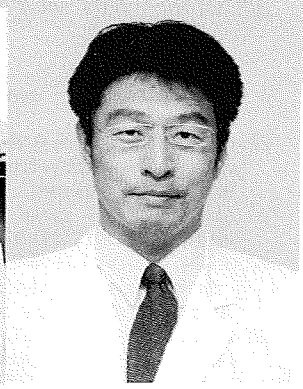

一言アピール

尿道括約筋不全による腹圧性尿失禁は、女性骨盤底機能障害、前立腺癌に対する根治的前立腺摘除術後、および神経因性膀胱においてみられ、その治療需要は極めて大きいにもかかわらず、有効な治療法が確立されていない。我々は、自己採取脂肪由来幹細胞を用いた尿道括約筋再生による臨床治療応用を目指している。
また、腎移植のドナー不足の問題点を解消するために、腎微小循環から尿細管上皮細胞再生にホーカスを当て、尿中落下細胞から培養した尿細管上皮前駆細胞を中心とした包括的臨床腎臓再生技術を開発している。

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研究のキーワード

尿失禁、尿中落下細胞、尿細管上皮前駆細胞、SP 細胞、末梢血幹細胞、脂肪由来幹細胞

保有技術・機器

【保有技術】・毛細血管血流時空間解析
【主な機器】生体顕微鏡、腎臓拡大内視鏡

主な特許・論文・著書

【主な特許】・特願 2006-216234　低血清培養による脂肪由来幹細胞治療（尿失禁、腎障害、創傷治癒、骨粗鬆症、下肢虚血）
・特願 2006-114152　尿中落下細胞からの尿細管前駆細胞の培養と腎障害治療
・特願 2003-375321　末梢血幹細胞による腎機能障害治療
・特開 2003-329934　生体顕微鏡における光学経路

【主な論文】・Kondo A, Isobe Y, Kimura K, Kamihira O, Matsuura O, Gotoh M, Ozawa H: Efficacy, safety and hospital costs of tension-free vaginal tape and pubovaginal sling in the surgical treatment of stress incontinence. *Obstet Gynaecol Res*, 32:539-544, 2006
・Homma Y, Yoshida Y, Seki N, Yokoyama O, Kakizaki H, Gotoh M, Yamanishi T, Yamaguchi O, Takeda M and Nishizawa O: Symptom assessment tool for overactive bladder syndrome-overactive bladder symptom score. *Urology*, 68: 318-323, 2006
・Gotoh M, Yoshikawa Y and Ohshima S: Pathophysiology and subjective symptoms in women with impaired bladder emptying. *Int J Urol*, 13:1053-1057, 2006
・Ohkawa A, Kondo A, Takei M, Gotoh M, Ozawa H, Kato K, Ohhashi T, Nakata M: Tension-free vaginal tape surgery for stress urinary incontinence: A prospective multicentered study in Japan. Int J Urol, 13:738-742, 200

研究 01　尿失禁の再生治療

概要

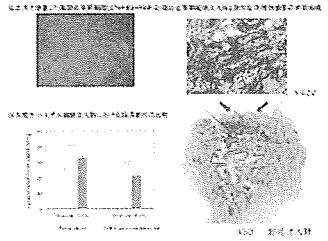
腹圧性尿失禁は、女性骨盤底機能障害、前立腺癌に対する根治的前立腺摘除術、神経因性膀胱などにおける尿道括約筋障害によってみられ、潜在患者は極めて多いにもかかわらず、適切な治療が得られず、患者の QOL 低下を引き起こしていることが多い。現在有効な薬物治療はなく、女性における括約筋不全による腹圧性尿失禁に対しては TVT スリングなどの外科的治療が広く行われているが、根治的前立腺摘除術後や神経因性膀胱に対しては有効な治療法がなく、新しい治療の開発が待たれている。尿道括約筋再生治療は、原因疾患にかかわらず括約筋障害にもとづく腹圧性尿失禁に対して有望な治療方法である。さらに、自己脂肪由来幹細胞は採取が容易であるとともに、その括約筋への注入は経尿道的内視鏡下に容易に行うことができ、脂肪由来幹細胞による括約筋再生治療は、低侵襲で実現可能性が高く、また治療による QOL 改善効果へのインパクトが極めて大きいものである。

この研究の新規性・独創性

低血清培養による小型でヒト、ラットの比較的均一な脂肪由来幹細胞の培養に成功した。麻酔下に除神経した尿失禁モデルラットの膀胱頚部に局所注入した２週間後、尿道抵抗を反映する leak point pressure（膀胱に生理食塩水を注入し尿が漏れる時の膀胱内圧を測定する）がコントロール群に比して有意に高い、すなわち尿失失禁を改善せしめる所見を得た。その部位の病理組織では尿道周囲にコラーゲン繊維を反映するマッソン染色される細胞塊のこぶが形成され、尿道内圧が上昇した一つの原因と考えられた。この研究は特許出願しており、名古屋大学独自のものである。

産学連携を目指した応用研究

尿失禁の新しい治療として将来が期待される。尿失禁薬剤を開発している薬品会社の共同開発を求める。



研究 02　腎臓の再生治療

概要

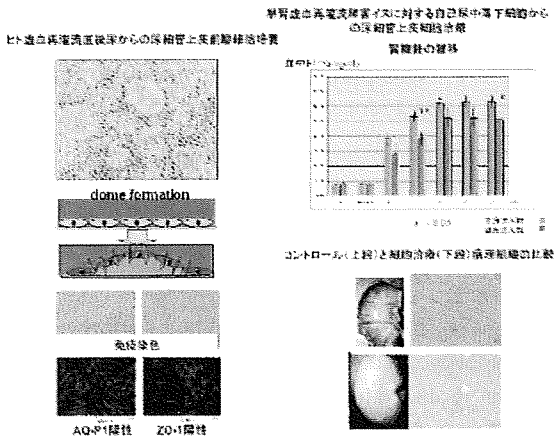
腎再生のマテリアルとして尿細管上皮細胞の再生を中心に 1) 尿中落下細胞からの尿細管上皮細胞（腎組織幹細胞）の培養治療、2) 低血清培養による脂肪由来幹細胞 3）末梢血幹細胞 4)HUVEC を用いて腎障害を軽減することを明らかにした。また独自に開発した生体顕微鏡でその微小循環環境を解明した。

この研究の新規性・独創性

1) については、臨床において生体腎移植、死体腎移植そして腎血管を一時的に遮断して行う腎部分切除の血流再開直後の尿から落下細胞を採取、ドーム形成を有する尿細管上皮前駆細胞（腎組織幹細胞）の一次培養に初めて成功した。その細胞は 0.33% と高率にも SP 細胞を含んでいた。また、単腎イヌ虚血再灌流障害モデルにこの細胞を皮膜下投与を行い腎保護作用を明かにし、細胞治療の可能性を示唆した。この研究は上皮性細胞治療で 2) は間葉系細胞治療であり、1）2）を組み合わせた上皮—間葉系治療も今後行う予定である。
1-3 は特許出願しており、名古屋大学独自のものである。
腎機能障害の新しい治療として将来が期待される。

産学連携を目指した応用研究

腎機能障害の新しい治療として将来が期待される。透析装置または腎保護作用の薬剤を開発している企業または薬品会社の共同開発企業を求める。



これまでの研究テーマ

- 膀胱微小循環障害の解明と治療薬剤の開発
- 腎微小循環障害の解明とその治療薬剤の開発
- 腎微小循環の酸化ストレスとバイオマーカの開発

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Original Article: Clinical Investigation

Periurethral injection of autologous adipose-derived stem cells for the treatment of stress urinary incontinence in patients undergoing radical prostatectomy: Report of two initial cases

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Objectives: To report a novel cell therapy using autologous adipose tissue-derived stem cells (ADSC) for stress urinary incontinence caused by urethral sphincteric deficiency and the outcomes in two initial cases undergoing periurethral injection of stem cells for the treatment of urinary incontinence after radical prostatectomy.

Methods: Two patients with moderate stress incontinence after radical prostatectomy were enrolled. After liposuction of 250 mL of adipose tissue from the abdomen, we isolated ADSC from this tissue by using the Celution system. Subsequently, the isolated ADSC and a mixture of stem cells and adipose tissue were transurethrally injected into the rhabdosphincter and submucosal space of the urethra, respectively. Short-term outcomes during a 12-week follow-up were assessed by a 24-h pad test, a validated patient questionnaire, urethral pressure profile, transrectal ultrasonography, and magnetic resonance imaging.

Results: Urinary incontinence progressively improved after 2 weeks of injection up to 12 weeks in terms of decreased leakage volume in a 24-h pad test, decreased frequency and amount of incontinence, and improved quality of life as per the questionnaire. In urethral pressure profile, both maximum urethral closing pressure and functional profile length increased. Ultrasonography and magnetic resonance imaging showed sustained presence of the injected adipose tissue. Enhanced ultrasonography showed a progressive increase in the blood flow to the injected area. No significant adverse events were observed peri- and postoperatively.

Conclusion: This preliminary study showed that periurethral injection of the autologous ADSC is a safe and feasible treatment modality for stress urinary incontinence.

Key words: adipose-derived stem cells, injection, radical prostatectomy, stress urinary incontinence, urethra.

Introduction

Prostate cancer is one of the most highly prevalent malignancies among the male population, and radical prostatectomy has been used worldwide as a standard treatment method for localized prostate cancers. Urinary incontinence is a distressing complication of radical prostatectomy and is associated with the functional impairment of the external urethral sphincter. The incidence of urinary incontinence after radical prostatectomy varies greatly across reported literature. Carlson *et al.*[1] summarized the reported rates of postprostatectomy incontinence in a large series and found that it varied from 2% to 90%. The large discrepancies in the reported incidence rates of postprostatectomy incontinence are attributed to a variety of factors such as definitions of continence, method of data collection, and the duration of follow-up; nonetheless, a substantial number of patients are known to suffer from long-lasting moderate to severe incontinence after radical prostatectomy.[2] Although a variety of treatment modalities have been attempted,[3] there has not been any well established treatment for postprostatectomy incontinence. Hence, there is an extreme need to develop a new minimally invasive and effective treatment modality for postprostatectomy incontinence.

Cell therapy for the regeneration of injured tissues has recently been extensively investigated at an experimental level, and its clinical application in a variety of fields has also been in progress. Mesenchymal stem cells (MSC) are multipotent adult stem cells that can proliferate in culture and are able to differentiate into a variety of mesenchymal cell phenotypes.[4–8] Thus far, MSC have been mainly harvested from bone marrow, a tissue source that has many

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limitations. These include donor-site morbidity in the bone marrow, which limits the amount of marrow that can be obtained,[9,10] MSC represent less than 0.01% of all nucleated bone marrow cells in healthy volunteers,[11,12] and an extended culture time is required to obtain therapeutic cell doses of MSC by using the *ex vivo* cell expansion method.

It has recently been shown that adipose tissue contains multipotent cells that are similar to MSC,[13,14] and the abundance of stem cells in the adipose tissue is 100-fold higher than that in the bone marrow. This finding has generated major interest because, unlike bone marrow cells, adipose tissue can be easily and safely harvested in large quantities with minimal morbidity, making it an appealing source for cell therapy. It has been shown that adipose-derived stem cells (ADSC) are multipotent and differentiate into several cell types[4,7,8,15] such as bone, cartilage, fat, nerves, blood vessels, and contractile cells with striated muscle cell[6] or cardiomyocyte features.[6,16] In addition, it has been shown that cultured ADSC secrete a variety of angiogenesis-related cytokines, such as hepatocyte growth factor and vascular endothelial growth factor.[17]

The Celution system (Cytori Therapeutics, San Diego, USA) is a commercially available device that allows the isolation of ADSC fractions from human adipose tissue in a short time.[18] This instrument allows the isolation of therapeutic doses of autologous ADSC after liposuction, obviating the need for culture. We developed a novel cell therapy for stress urinary incontinence (SUI) caused by urethral sphincter deficiency; the cell therapy included periurethal injection of autologous ADSC. In the present study, we report our experience of two initial patients with SUI undergoing this therapy after radical prostatectomy and focused on the procedure and short-term outcomes.

Methods

The present study was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine and written informed consent was obtained from both the patients.

Patients

In the present study, two patients with SUI after radical prostatectomy were enrolled. The inclusion criteria for the patients was as follows: persistence of moderate to severe urinary incontinence for more than 2 years after surgery and no evidence of recurrence or metastasis of prostate cancer, with undetectable levels of prostate-specific antigen (<0.008 ng/mL). The first patient (Case 1) was 75 years-of-age, had undergone radical prostatectomy 3 years earlier with a pathological stage of T2N0M0 and had undetectable levels of prostate-specific antigen at enrolment. He had moderate SUI without urgency. The second patient (Case 2) was 83 years-of-age, had undergone radical prostatectomy 7 years earlier with a pathological stage of T1cN0M0 and had undetectable levels of prostate-specific antigen at enrolment. He had moderate SUI without urgency and had undergone periurethral injection of collagen (Contigen, Bard, Covington, GA, USA) 3 years earlier, which was uneventful. Both patients had no pharmacologic treatment for SUI before and after the present treatment.

Harvesting adipose tissue (liposuction)

Under general anesthesia, 250 mL of adipose tissue was harvested from the anterior abdominal wall by making two 3-mm incisions. An 18-G Becker cannula with a 50 mL syringe was used as a collecting device; Ringer's lactate was first infused in the subcutaneous layer, then the adipose tissue was harvested. The suctioned adipose tissue contained in the saline was allowed to stand for settling the blood and cellular debris; adipose tissue floated to the top of the mixture.

Isolation of ADSC

ADSC were isolated from the harvested adipose tissue by using the Celution system[18]. Briefly, adipose tissue was introduced into the Celution cell-processing device, which automatically and aseptically extracts and concentrates the mononuclear fraction of adipose tissue and removes unwarranted or deleterious cells, cell and matrix fragments. It required around 1 h to process 250 mL of liposuction tissue. The final concentrated cell output collected using the Celution system was counted using a NucleoCounter (Chemometec, Allerod, Denmark), which exclusively detected nucleated cells. By using the Celution system, we could finally obtain a 5 mL solution containing concentrated ADSC.

Periurethral injection of ADSC

Subsequent to liposuction and isolation of ADSC, transurethral endoscopic injection of ADSC was carried out. For periurethral injection of ADSC, two distinct formulations were produced: 1 mL of the isolated ADSC fraction alone was preserved for direct injection and another 4 mL of the fraction was mixed with intact autologous adipose, producing a total of 20 mL of this combined solution.

A 22-Fr rigid nephroscope was used for injecting the processed ADSC solution. The nephroscope was inserted into the urethra. Under endoscopic vision, a puncture needle was passed through the nephroscope into the urethra at the region of the external urethral sphincter. The puncture needle with a thickness of 18 gauge, a length of 35 cm and graduated in centimeters was specially ordered. After puncturing the urethra at the region of the external urethral

sphincter under endoscopic vision, the ADSC solution was injected. Initially, a 1 mL solution was injected at a depth of 5 mm into the rhabdosphincter at 5 and 7 o'clock positions. Subsequently, 20 mL of the formulation containing ADSC and adipose tissue was equally injected into the submucosal spaces at 4, 6, and 8 o'clock positions to facilitate complete coaptation of the urethral mucosa by the bulking effect. After the solution was injected, a 6-Fr urethral balloon catheter was placed and was removed the next day to allow micturition.

Outcome measures

The amount of incontinence was evaluated by a 24-h pad test. The amount of leakage was calculated by subtracting the dry weight of the pad from that of the wet pad. The total daily leakage amount was calculated. The 24-h pad test was consecutively repeated for 4 days for each evaluation period. The subjective symptoms and quality of life were evaluated using a validated disease-specific questionnaire – the International Consultation on Incontinence Questionnaire-Short Form (ICIQ-SF)[19,20] (Appendix). In the ICIQ-SF, the therapeutic effects in terms of frequency of urinary incontinence (0–5 point scores), amount of leakage (0–6 point scores) and impact on everyday life (0–10 point scores) were examined, and the total score ranging from 0 to 21 points was calculated. A high score indicated an unfavorable condition. These parameters were assessed at baseline and repeated 2, 4, 8, and 12 weeks after treatment.

The urethral sphincter function was objectively assessed by measuring the urethral pressure profile, using urodynamic equipment (MMS, Enschede, the Netherlands). Maximum urethral closing pressure (MUCP) and functional profile length (FPL) were measured at baseline, and 2 and 12 weeks after treatment.

The condition of the urethra after the administration of ADSC was monitored by transrectal ultrasonography. In addition, the blood flow to the area where ADSC were injected was assessed by contrast-enhanced transrectal ultrasonography by intravenously injecting perflubutane.[21] The morphological condition of the injected area was monitored by magnetic resonance imaging (MRI). These imaging examinations were carried out at 4 days, and 4 and 12 weeks after treatment.

Results

Liposuction from the abdomen was carried out without significant morbidity and 250 mL of adipose tissues could be harvested. The isolated adipose tissue solution contained 2.38×10^7ADSC (2.18×10^7 viable cells) and 3.20×10^7 (2.87×10^7 viable cells) in cases 1 and 2, respectively.

The urethral lumen at the region of the external urethral sphincter remained open, as observed by endoscopy, whereas the urethral lumen completely closed after the periurethral injection (Fig. 1).

Urinary incontinence disappeared within 1 week after receiving the injection, but it deteriorated thereafter. However, urinary incontinence progressively improved after 2 weeks of injection up to 12 weeks. This improvement in urinary incontinence was observed in both the cases (Tables 1,2). At 12 weeks after injection, urinary incontinence improved in terms of leakage volume measured by a 24-h pad test. Assessment of subjective symptoms and quality of life on the basis of the ICIQ-SF questionnaire showed similar improvement. Sphincteric function of the urethra was improved in both the cases, in terms of increased MUCP and FPL (Tables 1,2).

After the urethral catheter was removed, both the patients could void without significant residual urine. Neither of the patients complained of voiding symptoms. Uroflowmetry did not show significant voiding dysfunction or an increase in the amount of residual urine (Tables 1,2).

Transrectal ultrasonography morphologically showed the presence of the injected adipose tissue in the lateral and posterior regions of the urethral lumen; this tissue could also be located at 12 weeks after injection. In addition, enhanced ultrasonography showed a sequential increase in the blood

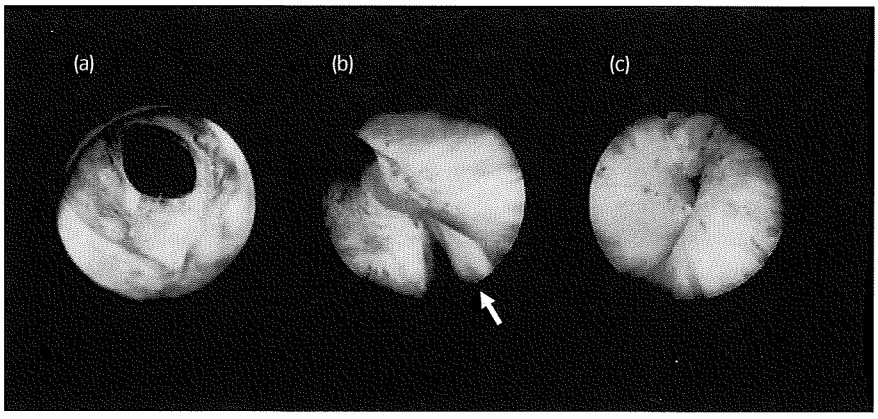

Fig. 1 Endoscopic findings of periurethral injection of adipose-derived stem cells in Case 1. (a) Before injection, the urethral lumen at the region of the external urethral sphincter was open. (b) An 18-G needle was injected into the urethra at 4 o'clock of the region of the external urethral sphincter (the arrow shows a puncture needle). (c) After completing the injection, the urethral lumen was closed and complete coaptation of the urethral mucosa was obtained.

Table 1 Clinical outcome in Case 1

	Baseline	2 Weeks	4 Weeks	8 Weeks	12 Weeks
24-h pad test (g) during 4 days (mean)	40/35/25/40 (35.0)	39/21/10/28 (24.5)	14/0/0/6 (5.0)	15/0/0/15 (7.5)	9/0/0/6 (3.8)
MUCP (cmH_2O)	28	25	–	–	43
FPL (mm)	14	20	–	–	32
ICIQSF (frequency of leakage)	4	4	3	2	2
ICIQSF (amount of leakage)	4	2	2	2	2
ICIQSF (QOL)	4	3	3	3	3
ICIQSF (total score)	12	9	8	7	7
Qmax	17.8	8.0	–	–	20.5
Postvoid residue (mL)	30	38	–	–	0

FPL, functional profile length; ICIQSF, International Consultation on Incontinence Questionnaire-Short Form; MUCP, maximum urethral closing pressure; Qmax, maximum flow rate; QOL, quality of life.

Table 2 Clinical outcome in Case 2

	Baseline	2 Weeks	4 Weeks	8 Weeks	12 Weeks
24-h pad test (g) during 4 days (mean)	76/22/40/38 (44.0)	30/20/20/23 (23.3)	30/25/25/15 (23.8)	23/18/16/19 (19.0)	20/19/18/16 (18.3)
MUCP (cmH_2O)	21	23	–	–	36
FPL (mm)	17	22	–	–	27
ICIQSF (frequency of leakage)	5	2	3	3	3
ICIQSF (amount of leakage)	4	2	2	2	2
ICIQSF (QOL)	10	3	3	3	3
ICIQSF (total score)	19	7	8	8	8
Qmax	9.7	13	–	–	8.1
Postvoid residue (mL)	20	27	–	–	10

FPL, functional profile length; ICIQSF, International Consultation on Incontinence Questionnaire-Short Form; MUCP, maximum urethral closing pressure; Qmax, maximum flow rate; QOL, quality of life.

flow to the area where ADSC were injected, which was maintained during the entire follow-up period (Fig. 2). MRI showed a bulking effect at the site of adipose tissue injection, which persisted even at 12 weeks after injection (Fig. 3).

No significant adverse event was noted throughout the liposuction and ADSC injection procedures. No severe side-effects such as pelvic pain, inflammation, or de novo urgency were observed after the operation in both the cases during the postoperative follow-ups.

Discussion

ADSC have been successfully used in a variety of indications in humans, including the treatment of Crohn's disease-associated fistulas,[22] Osteogenesis imperfecta,[23] and for breast augmentation and reconstruction after partial mastectomy.[24] Characterization and safety of ADSC isolated by the Celution system were reported in a basic investigation.[18] To explore the safety and feasibility of ADSC transplantation in patients with myocardial infarction, the first-in-man randomized controlled trial is currently in progress in the Netherlands.[16] The cell therapy in the present study is the first attempt to use ADSC for treating SUI.

Before applying this new therapeutic technique to humans, we carried out several animal experiments using rats to confirm the effect of the periurethral injection of ADSC on the urethral resistance and sequential changes of the injected rat ADSCs.[25] Cultured rat ADSC were injected into the proximal urethra of rats after bilateral transection of the pelvic nerves. Bladder leak point pressure was measured 4 weeks after injection of ADSC, GAX-collagen or vehicle. Leak point pressure was significantly higher in the rats undergoing ADSC injection as compared with those undergoing injection of collagen or vehicle. Additionally, green fluorescent protein (GFP)-expressing cultured ADSC

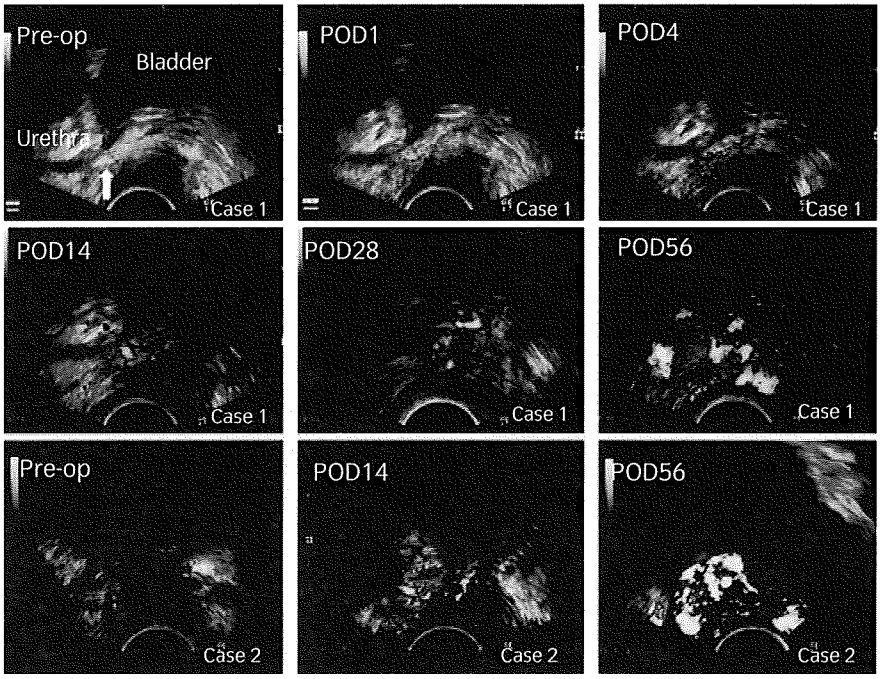

Fig. 2 Contrast-enhanced transrectal ultrasonography to assess the blood flow of the periurethral area after adipose-derived stem cells (ADSC) injection. The bladder and urethra were visualized as a sagittal section. The blood flow around the injected area, visualized as orange color, was progressively increased in both Case 1 and 2 after the injection of ADSC. POD, postoperative day.

obtained from male GFP rats were injected into the urethra of female nude rats. Four weeks after the injection, anti-GFP antibody-positive cells were abundantly stained at the region of ADSC injection. Furthermore, 12 weeks after the injection, although in a small proportion, alpha smooth muscle actin (SMA)-positive cells were stained in the merged distribution with the GFP expressing ADSC, suggesting possible differentiation of ADSC into smooth muscle cells. The results of these preliminary animal experiments support the present clinical outcomes, such as progressive improvement of sphincteric function and incontinence. Zeng *et al.*[26] also reported the feasibility of ADSC use as an improvement in leak point pressure and urethral function of SUI when animals were injected with ADSC in conjunction with hepatocyte growth factor-impregnated microspheres.

Recently, cell therapy using autologous adult muscle-derived stem cells has been developed for treating SUI. A small muscle biopsy sample was obtained from the upper arm and cultured to harvest two types of autologous muscle-derived cells: myoblasts and fibroblasts. The muscle-derived stem cells were injected transurethrally into the urethra under continuous monitoring. Carr *et al.*[27] reported the outcomes of 1-year follow-up of autologous muscle-derived stem cell injection to treat eight women with stress incontinence. With a mean follow-up period of 16.5 months, SUI improved in five patients, with one achieving total continence, and no serious adverse events were noted. The present treatment strategy has an important advantage over the use of muscle-derived stem cells. Because adipose tissue contains abundant multipotent stem cells, as well as key mature cells and progenitor cells, therapeutic levels of ADSC can be obtained rapidly using the Celution system. In the present study, a sufficient number of stem cells could be isolated from each patient using this system. Unlike other cell therapy strategies, the treatment is totally autologous, requires no cell culture and is carried out in the context of a single surgical procedure.

Periurethral injection of autologous fat was previously investigated in female patients with SUI; adipose tissue harvested from the abdomen was transurethrally injected into the submucosal layer under endoscopic vision. Although the injected adipose tissue could sustain a bulking effect, its efficacy was reported to be poor. In a randomized controlled trial that compared the efficacy of fat injection with that of a placebo injection (saline), the researchers found that the improvement rate after fat injection was poor (22%) at 3 months, with no difference than that produced by the placebo.[28] These findings suggested that mature adipocytes were unable to survive at the injected site.

In the periurethral injection of ADSC, it has been suspected that a variety of mechanisms are involved in the ADSC-mediated improvement of the sphincteric function. A similar clinical course in both the cases implies the involvement of specific factors that can suggest the mechanisms underlying the treatment strategy, such as urinary incontinence that disappeared within a week after injection then deteriorating subsequently, progressively improved thereafter up to 12 weeks after the injection. A bulking effect produced by the injected adipose tissue fraction mixed with ADSC is of primary importance. The injected adipose tissue fraction, which was processed to isolate ADSC, contained 30% of lactated Ringer's solution. Absorption of the

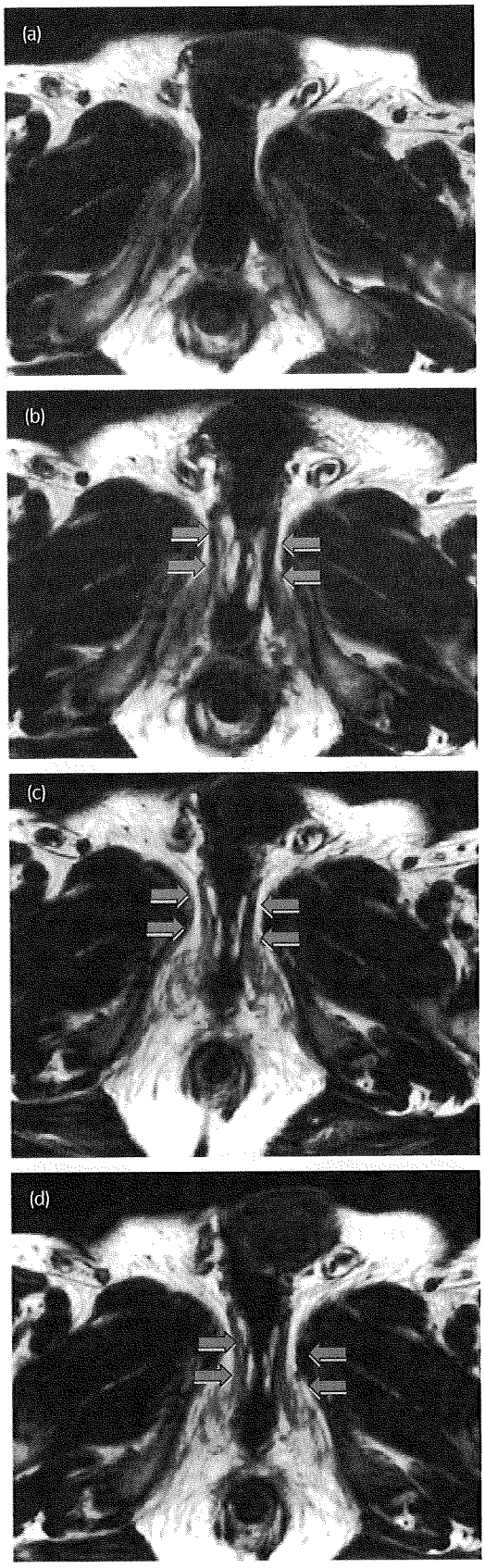

Fig. 3 Magnetic resonance imaging around the urethra before and after the adipose-derived stem cells (ADSC) injection. The urethra in Case 1 was visualized as a cross-section on magnetic resonance imaging. (a) Before injection, (b) 4 days after injection, (c) 4 weeks after injection, and (d) 12 weeks after injection. The arrows show the adipose tissue injected with ADSC. At 4 weeks after injection, the volume of injected adipose tissues had decreased compared with that after 4 days after injection. However, the volume did not change thereafter and persisted at 12 weeks after injection.

solution could be responsible for the temporary deterioration in the patient's condition during the initial week. ADSC might have contributed to the progressive improvement in sphincteric function, which was shown in terms of increased MUCP and FPL, as well as decreased frequency and amount of urinary incontinence. Persistent bulking effect indicates the survival and growth of the injected adipose tissue, which could also be attributed to the presence of ADSC. ADSC might differentiate into mature adipose tissue and, possibly, into contractile cells. Previous studies on rats showed that cultured adipose-derived stem cells injected into the injured urethra differentiated into contractile cells with smooth muscle cell features.[29] Indirect effects of the injected ADSC might also be responsible for the improvement in the sphincteric function. Cultured ADSCs\ are known to secrete a large number of angiogenesis-related cytokines.[17] In the present study, increased blood flow to the injected area was confirmed by ultrasonography. The increased blood flow was not temporary, but was maintained throughout the follow-up period; this increased blood flow could be the result of the angiogenesis effect of the cytokines secreted by the injected ADSC. The increased blood flow might have a positive effect on the regeneration of the injected adipose tissue and impaired intrinsic sphincteric function.

Conclusions

The present preliminary study showed that periurethral injection of the autologous adipose-derived stem cells is a safe and feasible treatment modality for stress urinary incontinence. We could establish the clinical course and obtained excellent short-time outcomes in the two initial cases who underwent this cell therapy. Hence, we intend to increase the number of patients and confirm the long-term outcomes of this treatment modality.

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Appendix

ICIQ-SF

1. How often do you leak urine? *(Tick one box)*		
Never	☐	0
About once a week or less often	☐	1
Two or three times a week	☐	2
About once a day	☐	3
Several times a day	☐	4
All the time	☐	5

We would like to know how much you think leaks.

2. How much urine do you usually leak (whether you wear protection or not)? (Tick one box)		
None	☐	0
A small amount	☐	2
A moderate amount	☐	4
A large amount	☐	6

3. Overall, how much does leaking urine interfere with your everyday life?

Please ring a number between 0 (not at all) and 10 (a great deal)

0	1	2	3	4	5	6	7	8	9	10
Not at all										A great deal

ICIQ score: sum scores 1 + 2 + 3 ☐

Change in Contralateral Renal Parenchymal Volume 1 Week After Unilateral Nephrectomy

Yasuhito Funahashi, Ryohei Hattori, Tokunori Yamamoto, Osamu Kamihira, Yoshie Moriya, and Momokazu Gotoh

OBJECTIVES To measure the contralateral renal parenchymal volume (RPV) before and after nephrectomy and investigate the factors influencing compensatory hypertrophy. Unilateral nephrectomy induces compensatory hypertrophy in the contralateral kidney.

METHODS From December 2003 to January 2008, 142 patients undergoing nephrectomy were enrolled in this study. All patients underwent preoperative technetium-99m dimercaptosuccinic acid renal scintigraphy. The percentage of technetium-99m dimercaptosuccinic acid uptake in the resected kidney was 37.2% ± 15.3%. Contrast-enhanced computed tomography was performed preoperatively and 1 week and 6 months postoperatively, and RPV was calculated as the normally functioning tissue, excluding tumors or nonenhanced areas.

RESULTS The mean RPV of the remaining kidney was 164.2 cm^3 preoperatively and 184.1 and 178.8 cm^3 at 1 week and 6 months postoperatively, respectively. Multivariate regression analysis revealed that the increase in RPV was positively associated with the percentage of technetium-99m dimercaptosuccinic acid uptake in the resected kidney ($P < .001$) and negatively associated with patient age ($P = .008$). Logistic regression analysis showed that the group with an RPV increase of <15% had a 4.1-fold increased risk of a 10% decrease in the glomerular filtration rate during the next 6 postoperative months compared with the risk in the group with an RPV increase of ≥15% ($P = .004$).

CONCLUSIONS The change in contralateral RPV occurred during the first week after nephrectomy and remained stable for ≥6 months. The change in RPV increased when the removed kidney had greater function and decreased with increasing patient age. The risk of progression to renal insufficiency can be predicted according to the change in RPV. UROLOGY 74: 708–712, 2009. © 2009 Elsevier Inc.

The number of patients undergoing unilateral nephrectomy for renal malignancy has increased because of the aging population and the widespread use of various imaging modalities such as ultrasonography, computed tomography (CT), and magnetic resonance imaging. Because these patients tend to be elderly, it is important to predict the postoperative renal function when selecting the appropriate surgical procedure, whether radical or partial nephrectomy. Many reports have described the functional adaptation of the remaining kidney after unilateral nephrectomy. The glomerular filtration rate (GFR) increases to 60%-80% of the preoperative level within several weeks postoperatively and then stabilizes or increases very slightly for >10 years after nephrectomy.[1,2] Advanced age, female sex, hypertension, and proteinuria have been reported as negative factors for functional adaptation.[3-5] Not only the function, but also the size of the remaining kidney is known to be a factor in the ability to compensate for the loss of functioning nephrons. Most of the renal growth occurs in the cortex, particularly in the proximal tubules, and appears to be of a hypertrophic (an increase in cell size), rather than a hyperplasic (an increase in cell number) nature.[6] However, few reports have described the sequential changes in renal volume after unilateral nephrectomy or the correlation between hypertrophy and renal function. The evaluation of contiguous CT slices is a reliable, objective, and reproducible method of assessing the renal volume.[7] The kidney volume has been shown to correlate well, although indirectly, with the number of functioning nephrons found in autopsy studies,[8] and the correlation between the renal parenchymal volume (RPV) calculated using CT images and the single-kidney GFR is significant.[9] In the present study, we measured normally functioning renal tissue, excluding tumors, cystic lesions, and low-density areas,

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using CT images, and investigated the factors influencing compensatory hypertrophy after unilateral nephrectomy.

MATERIAL AND METHODS

Patient Population

From December 2003 to January 2008, a total of 202 patients underwent unilateral nephrectomy at Komaki City Hospital. Preoperative CT images were not available for 18 patients, and contrast media could not be administered to 12 patients because of low renal function or allergy to iodinated contrast medium. Furthermore, 30 patients underwent chemotherapy. These 60 patients were excluded from the present study. The remaining 142 patients, including 69 with renal cell carcinoma, 51 with urothelial carcinoma, 16 with an atrophic kidney, 5 kidney donors, and 1 with liposarcoma were enrolled. Of these patients, 104 were men and 38 were women. Their mean age was 62.8 ± 13.0 (mean ± SD) years (range 22-93). A total of 53 patients were receiving medications for hypertension and 10 for diabetes.

Imaging Procedures

The patients were injected with 74 MBq technetium-99m dimercaptosuccinic acid (DMSA). After 2 hours, posterior images were obtained using a Hitachi/Philips SKY Light gamma camera (Japan) equipped with a low-energy, general-purpose collimator. The regions of interest were drawn on the images for both kidneys showing the calculated percentage of split function. Of the 142 patients, 140 underwent preoperative DMSA renal scintigraphy.

Contrast-enhanced CT scans were obtained with a multislice 16-row unit (Sensation 16 Cardiac, Siemens, Germany) at a table speed of 20 mm/s and a slice thickness of 5 mm, with a 100-mL injection of intravenous iodinated contrast agent (flow 2 mL/s). The regions of interest were drawn around the kidneys, excluding the peripelvic fat and renal pelvis from the images by experienced radiologists who were unaware of the patients' clinical information. Functioning renal tissue was determined as the normally enhanced areas on the CT images, and tumors and cysts were excluded from the regions of interest. RPV was calculated using Volume software (Siemens, Germany), three-dimensional image reconstruction program. All patients underwent CT before unilateral nephrectomy. Also, 140 and 122 patients underwent CT at 1 week and 6 months postoperatively, respectively.

Serum creatinine was simultaneously determined, and the GFR was calculated using the revised modified diet in renal disease formula (GFR [mL/min/1.73 m^2] = 0.741 × 175 × $age^{-0.203}$ × $creatine^{-1.154}$ [× 0.742 for female sex]). The single GFR of the remaining kidney was calculated from the total GFR and the uptake ratio of the remaining kidney on DMSA renal scintigraphy.

Statistical Analysis

Continuous variables are presented as the mean ± SD and compared using Student's t test. One-way analysis of variance was used to test for an association between the percentage of DMSA uptake (%DMSA) in the resected kidney and the increase in contralateral RPV. The differences in the prevalence of hypertension and diabetes were evaluated using the χ^2 test. Multivariate stepwise regression analysis was used to test the relationship between the increased rate of remnant RPV and patient characteristics (sex, age, body surface area, %DMSA of resected kidney, preoperative GFR of remaining kidney, hypertension, and diabetes mellitus). Significant predictors of a GFR decrease were investigated using logistic regression analysis on the increased rate of the remnant RPV. All tests were 2-sided, and $P < .05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences software (SPSS, Chicago, IL).

Table 1. Patient characteristics

Characteristic	Value
Patients (n)	142
Sex	
Male	104
Female	38
Age (y)	62.8 ± 13.0
Pathologic findings	
Renal cell carcinoma	69
Urothelial carcinoma	51
Atrophic kidney	16
Renal transplant donor	5
Liposarcoma	1
Serum creatinine (mg/dL)	
Preoperatively	0.87 ± 0.24
6 mo postoperatively	1.15 ± 0.28
GFR (mL/min/1.73 m^2)	
Preoperatively	66.0 ± 18.3
Remaining kidney	
Preoperative	40.3 ± 11.9
6 mo postoperatively	47.8 ± 15.5
RPV of remaining kidney (cm^3)	
Preoperatively	164.2 ± 37.9
Postoperatively	
1 wk	184.1 ± 44.7
6 mo	178.8 ± 41.1
Preoperative RPV of resected kidney (cm^3)	120.4 ± 55.2

GFR, glomerular filtration rate; RPV, renal parenchymal volume.

RESULTS

The patient data are summarized in Table 1. The %DMSA in the resected kidney was 37.2% ± 15.3%. The preoperative serum creatinine level was 0.87 ± 0.24 mg/dL (range 0.43-1.76) and increased to 1.11 ± 0.30 mg/dL (range 0.49-1.98) at 1 week postoperatively. The mean GFR of the remaining kidney increased by 27.1% from 40.3 to 49.8 mL/min/1.73 m^2 at 1 week after nephrectomy ($P < .001$).

The preoperative RPV of the remaining kidney was 164.2 ± 37.9 cm^3 and that of the resected kidney was 120.4 ± 55.2 cm^3. The RPV of the remaining kidney increased by 12.1% to 184.1 ± 44.7 cm^3 ($P < .001$) and by 8.9% to 178.8 ± 41.1 cm^3 at 1 week and 6 months after surgery, respectively ($P < .001$).

The patients were divided into 5 groups according to the %DMSA in the resected kidney. The increase in

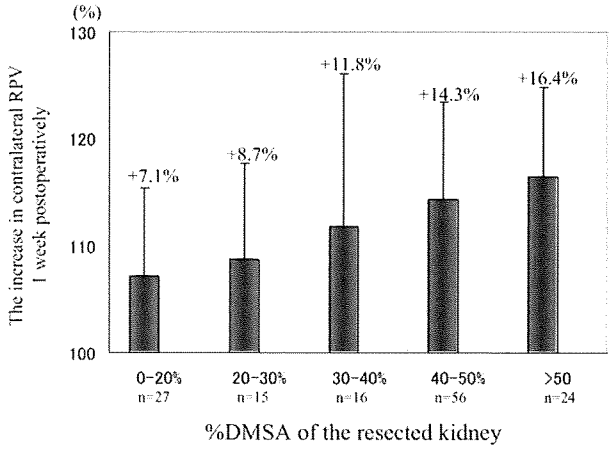

Figure 1. Correlation between preoperative percentage of technetium-99m dimercaptosuccinic acid uptake in resected kidney and contralateral renal parenchymal volume increase at 1 week postoperatively.

RPV was 7.1% in group 1 (%DMSA 0-20, n = 27), 8.7% in group 2 (%DMSA 20-30, n = 15), 11.8% in group 3 (%DMSA 30-40, n = 16), 14.3% in group 4 (%DMSA 40-50, n = 56), and 16.4% in group 5 (%DMSA >50, n = 24; Fig. 1). One-way analysis of variance detected statistically significant differences among the groups (P = .003).

Multivariate regression analysis revealed that the increase in RPV was positively associated with the %DMSA in the resected kidney (P < .001) and negatively associated with patient age (P = .008). Furthermore, no statistically significant association was noted for sex (P = .112), body surface area (P = .298), preoperative GFR of the remaining kidney (P = .452), or the presence of hypertension (P = .182) or diabetes mellitus (P = .060; Table 2).

For subanalysis, the study population was stratified into 2 groups according to the increase in RPV at 1 week to determine its relationship with renal function deterioration. Group 1 included patients with an RPV increase of <15% and group 2 those with an RPV increase of ≥15% (Table 3). The patients in group 1 were significantly older and had a lower %DMSA in the resected kidney compared with group 2 patients. At 6 months postoperatively, 37.2% (35/94) and 13.0% (6/46) of patients in groups 1 and 2, respectively, exhibited a >10% decrease in the estimated GFR compared with the corresponding values at 1 week postoperatively. Logistic regression analysis showed that the relative risk of decrease in renal function in group 1 patients was 4.1-fold (95% confidence interval 1.6-10.7; P = .004) greater than that in group 2 patients.

COMMENT

Unilateral nephrectomy in a patient with 2 normally functioning kidneys induces the immediate adaptation in both function and structure in the remaining kidney. Anderson et al.[4] reported that the effective renal plasma flow increased by 32.5% at 1 week, 30.1% at 1 year, and 37.2% at 10 years compared with the baseline preoperative values after donor nephrectomy. Indudhara et al.[10] evaluated renal transplant donors with fibromuscular dysplasia of the renal arteries and reported that the effective renal plasma flow in the remaining kidney had increased by 25% on postoperative day 5 and by 29% at 1 year postoperatively. With regard to the kidney volume, Mullerad et al.[11] evaluated renal hypertrophy after unilateral nephrectomy using DMSA renal scintigraphy. The mean age of their study population was 61.5 years, and the mean uptake ratio in the resected kidney was 40.74%. They reported that the RPV increased postoperatively by 11% ± 17.5%. Our patients' characteristics were almost identical to those of their patients, and the increase in RPV was 12.1% at 1 week and 8.9% at 6 months. Although the resected kidneys had lower function than the remaining kidneys, their average contribution to the overall baseline renal function was significant enough to cause a compensatory change in the remaining contralateral kidney.

Previously, we investigated the compensatory RPV increase of the normal side after nephron-sparing surgery.[12] In that study, we reported that the RPV of the normal kidney had increased by 9.6% at 1 week and 9.3% at 6 months after surgery. The contribution of the renal function in the operated side to the total renal function was 49.5%. In the present study, when the function of the resected kidney was 40%-50%, the RPV of the remaining kidney increased by 14.3% at 1 week after unilateral nephrectomy, a much greater increase than that after nephron-sparing surgery.

In our present study of unilateral nephrectomy and in our previous study of nephron-sparing surgery, we showed that the RPV increases and reaches a plateau within 1 week. It is unlikely that rapid histologic reconstruction is completed within a brief period of 1 week. Many studies have reported on the hemodynamic and structural changes after unilateral nephrectomy. The control of renal blood flow involves a complex interaction between circulating hormones and several vasoactive humoral factors in combination with the autonomic nervous system

Table 2. Correlation between patient characteristics and increase in RPV 1 week after nephrectomy

Factor	*P* Value
Sex	0.122
Age	.008
Body surface area	.298
%DMSA of resected kidney	<.001
Preoperative GFR of remaining kidney	.452
Hypertension	.182
Diabetes mellitus	.060

%DMSA, percentage of technetium-99m dimercaptosuccinic acid uptake; GFR, glomerular filtration rate.

Table 3. Patient characteristics stratified by increase in RPV 1 week postoperatively

Characteristic	RPV Increase		*P* Value
	<15% (Group 1)	≥15% (Group 2)	
Patients (n)	94	46	
Sex			.309
Male	71	31	
Female	23	15	
Age (y)	64.6 ± 12.0	58.7 ± 14.1	.012
Hypertension			.205
Yes	55	32	
No	39	14	
Diabetes mellitus			.369
Yes	86	44	
No	8	2	
%DMSA of resected side (%)	33.3 ± 15.6	44.7 ± 11.9	<.001
Serum creatinine level (mg/dL)			
Preoperatively	0.90 ± 0.26	0.81 ± 0.21	.042
Postoperatively			
1 wk	1.09 ± 0.31	1.16 ± 0.30	.161
6 mo	1.15 ± 0.28	1.13 ± 0.28	.643
GFR (mL/min/1.73 m^2)			
Preoperatively	64.1 ± 19.3	70.2 ± 15.8	.067
Remaining kidney	41.4 ± 12.2	38.6 ± 11.2	.201
1 wk	51.3 ± 14.4	47.0 ± 13.4	.089
6 mo	47.2 ± 12.6	49.5 ± 20.2	.438

Abbreviations as in Tables 1 and 2.

and the autoregulatory capacity of the kidney. It has been reported that the renal blood flow increases within 2 days of the reduction in renal vascular resistance in nephrectomized rats.[13,14] Histologic analysis revealed the primary site of hypertrophy is the cortex, in which proximal tubular cell hypertrophy, collecting tubular cell enlargement, and glomerular mesangial expansion occur within weeks after nephrectomy.[15,16] The hypertrophic response to unilateral nephrectomy seems to be secondary to renal hemodynamic changes. Renal blood flow is difficult to measure noninvasively and, to our knowledge, no report has assessed sequential hemodynamic changes after radical nephrectomy. The increase in RPV at 1 week observed in our study would be a result of an enlarged vascular bed caused by increased renal blood flow. Limited to a short period, it might be possible to estimate the renal blood flow by calculating the RPV.

We found that the change in contralateral RPV at 1 postoperative week was favorable for renal function until ≥6 postoperative months. Thus, renal functional deterioration can be predicted by measuring the RPV at 1 week postoperatively. We suspect that kidneys with a lesser increase in size after contralateral nephrectomy might have poorer compensatory potential and thus a greater risk of renal insufficiency. It is still not clear whether an increase in renal blood flow and hypertrophy will ultimately lead to renal insufficiency. Some have reported that unilateral nephrectomy might negatively affect the remaining kidney,[11,17,18] and others have reported no increased risk for the development of renal failure.[19] A larger sample size and longer follow-up might be required to detect long-term changes in RPV and renal function in various clinical conditions.

CONCLUSIONS

The change in contralateral RPV occurred during the first week after nephrectomy and remained stable for ≥6 months. The change in RPV increases when the removed kidney has greater function and decreases with age. Whether the renal function at 6 months will deteriorate can be predicted by measuring the RPV at 1 week postoperatively.

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