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Effects of prednisolone on adriamycin-induced nephropathy in rats

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Summary in Japanese

慢性腎臓病（chronic kidney disease：CKD）は，腎機能が慢性にかつ進行性に悪化する病態の総称である．抗がん剤の一種であるアドリアマイシン（以下，ADR）を動物に投与することで，大量のタンパク尿を生じるネフローゼ症候群を誘導できることが知られている．このような背景から本研究では，ADR腎症ラットモデルについて，モデル確立（実験①）およびプレドニゾロンの薬効薬理試験（実験②）を実施した．

実験①では，Crlj:WI雄性ラットにADRを3パターンの用量（① 3 mg/kg，② 5 mg/kg，③ 10 mg/kg）で静脈内投与し，モデルを誘発した．なお3 mg/kg投与群のみ，初回ADR投与日の15日後に，再度2 mg/kgの用量でADRを投与した．ADR初回投与日をDay 1として，Day 42までの間に1回／週の頻度で採尿および採血を実施し，生化学的パラメータを測定した．

その結果，5 mg/kgの用量で以降の薬効評価試験を実施することとした．

実験②では，ADR初回投与日より1回／日の頻度で35日間，プレドニゾロンを1 mg/kgまたは5 mg/kgの用量にて経口投与した．その結果，プレドニゾロンを5 mg/kgの用量で投与した際に，有意ではないものの腎障害の改善が認められた．

また実験②では，Day 35に採取した新鮮尿，またはDay 36の解剖時に採取した腎臓の薄切切片からRNAを抽出し，糸球体障害マーカーとされるマイクロRNA（miR-26a）の測定を試みた．なお腎臓の薄切切片からのマイクロRNAの抽出は，レーザーマイクロダイシェクションを使用して糸球体部分のみを切り出し，糸球体特異的なRNAを抽出した．RT-PCR法で正常群，モデル対照群，プレドニゾロン投与群のmiR-26aの発現を測定したところ，正常群に対してモデル対照群のmiR-26aの発現が有意に低値を示し，（新鮮尿）プレドニゾロン投与群では有意では無いものの，モデル対照群に対して高値を示した（薄切切片）．

本研究の内容は慢性腎臓病に対する薬剤検討を実施するにあたり，有用であると考えられる．

Objective

Chronic kidney disease (CKD) is characterized by progressive and chronical kidney dysfunction. Although it can be categorized various types with pathogenesis, adriamycin (ADR) induced nephrotic syndrome is considered to be a classic rat model of CKD. By administration of ADR, glomerular filtration barrier damage and subsequently massive proteinuria are induced. In this study, we tried to prepare an ADR-induced nephropathy model in rats (**Experiment 1**) and to validate the usefulness of the model by administration of prednisolone (**Experiment 2**).

Materials and Methods

【Animal】

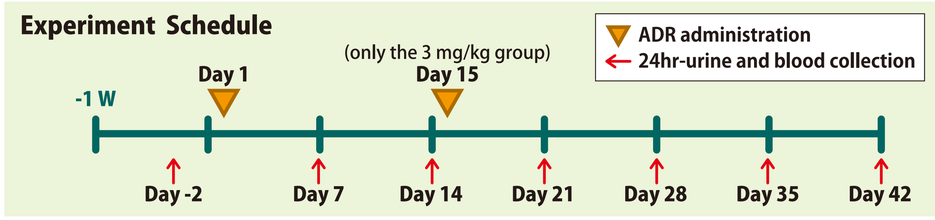
Crlj:WI, ♂, 7w (At the ADR administration)

Experiment 1: N=8×4 [Non-treatment, ADR-low dose group (3+2 mg/kg), ADR-middle dose group (5 mg/kg), ADR-high dose group (10 mg/kg)]

Experiment 2: N=8×4 [Non-treatment, Control group, prednisolone-low dose group (1 mg/kg), prednisolone-high dose group (5 mg/kg)]

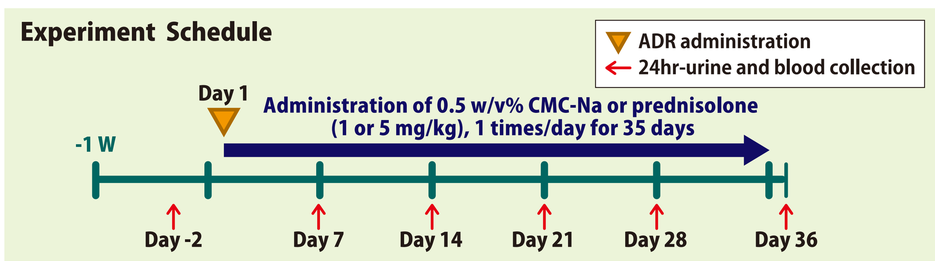
【ADR-induced nephropathy model in Experiment 1】

In the day 1, animals were administered ADR intravenously at 3, 5 or 10 mg/kg.
Only the 3 mg/kg group, 2 mg/kg of ADR was additionally administered at day 15.
24 hr-urine and blood were collected once weekly for 6 weeks (Day -2, 7, 14, 21, 28, 35 and 42) for urinalysis and blood chemistry-analysis.



【ADR-induced nephropathy model in experiment 2 and drug administration】

In the day 1, animals were administered ADR intravenously at 5 mg/kg. 24hr-urine and blood were collected once weekly for 6 weeks (Day -2, 7, 14, 21, 28 and 36) for urinalysis and blood chemistry-analysis.
In the day 35 only, fresh urine was collected for real-time PCR analysis. Prednisolone was administered orally at 1 or 5 mg/kg once a day for 35 days.
0.5 w/v% carboxymethyl cellulose-Na (0.5 w/v% CMC-Na) was administered orally to non-treatment and control group.
In the day 36, kidneys were collected and left kidney was used for histopathological analysis (H&E staining) and right kidney was used for real-time PCR analysis.



【Examination items】

(1) Urinalysis (Automated analyze method)
Total protein, Albumin (Alb), Creatinine (Cre)

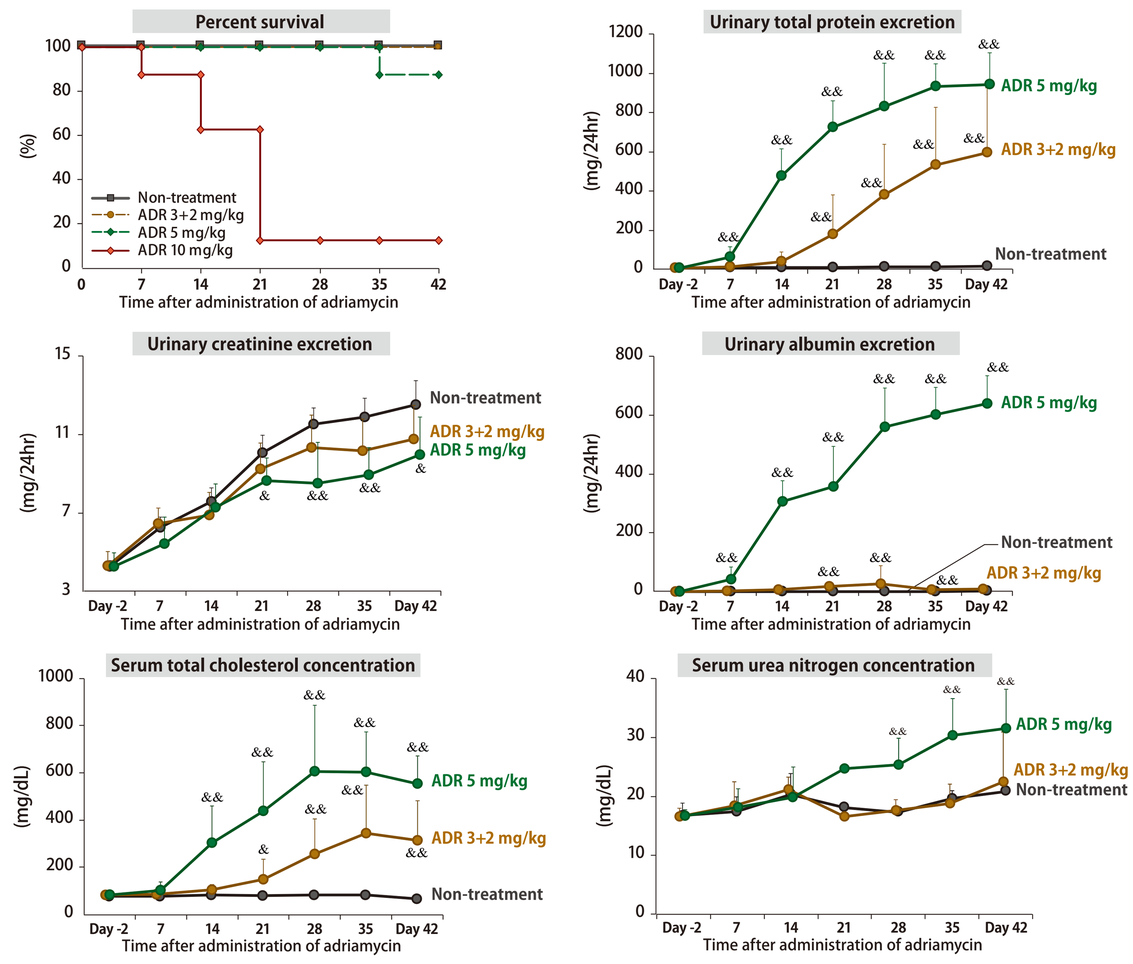
(2) Blood chemistry-analysis
Total cholesterol (T-CHO), Creatinine (Cre), Total protein, Albumin (Alb), Urea nitrogen (UN) (Data of Cre, Total protein and Alb are not shown.)

(3) Real-time PCR (RT-PCR)
Collected fresh urine was centrifuged (3,000×g for 15 minutes at r.t.) and supernatant (cell-free fraction) was transferred to a new tube. Transferred cell free-fraction was added Urine Conditioning Buffer (ZYMO RESEARCH CORP) and was centrifuged (3,000×g for 15 minutes at r.t.) to form a urine pelleted.
Collected left kidney were embedded with O.C.T compound in a routine method, and stained with toluidine blue. In the toluidine blue microscope slide, glomeruli (15 points per slide) were collected using laser microdissection (LMD, LMD7, Leica Microsystems GmbH).
Total RNA was extracted from urine pelleted or glomeruli section using the miRNeasy Mini Kit (QIAGEN). Complementary DNA was synthesized using the TaqMan™ advanced miRNA cDNA synthesis kit (Thermo Fisher scientific K.K.).
Real-time PCR was carried out with the TaqMan™ advanced miRNA assay (Thermo Fisher scientific K.K.) in the LightCycler 480 II instrument (Roche).
Endogenous controls was used miR-361-5p.
The relative expression of target gene (miR-26a) was calculated according to the next expression.

$$\Delta Cp(miR\text{-}26a) = Cp(miR\text{-}26a) - Cp(miR\text{-}361\text{-}5p)$$
$$\Delta\Delta Cp(miR\text{-}26a) = \Delta Cp(miR\text{-}26a) - \Delta Cp(\text{average of Non-treatment group})$$
$$\text{Relative expression to Non-treatment group} = 2^{-\Delta\Delta Cp(miR\text{-}26a)}$$

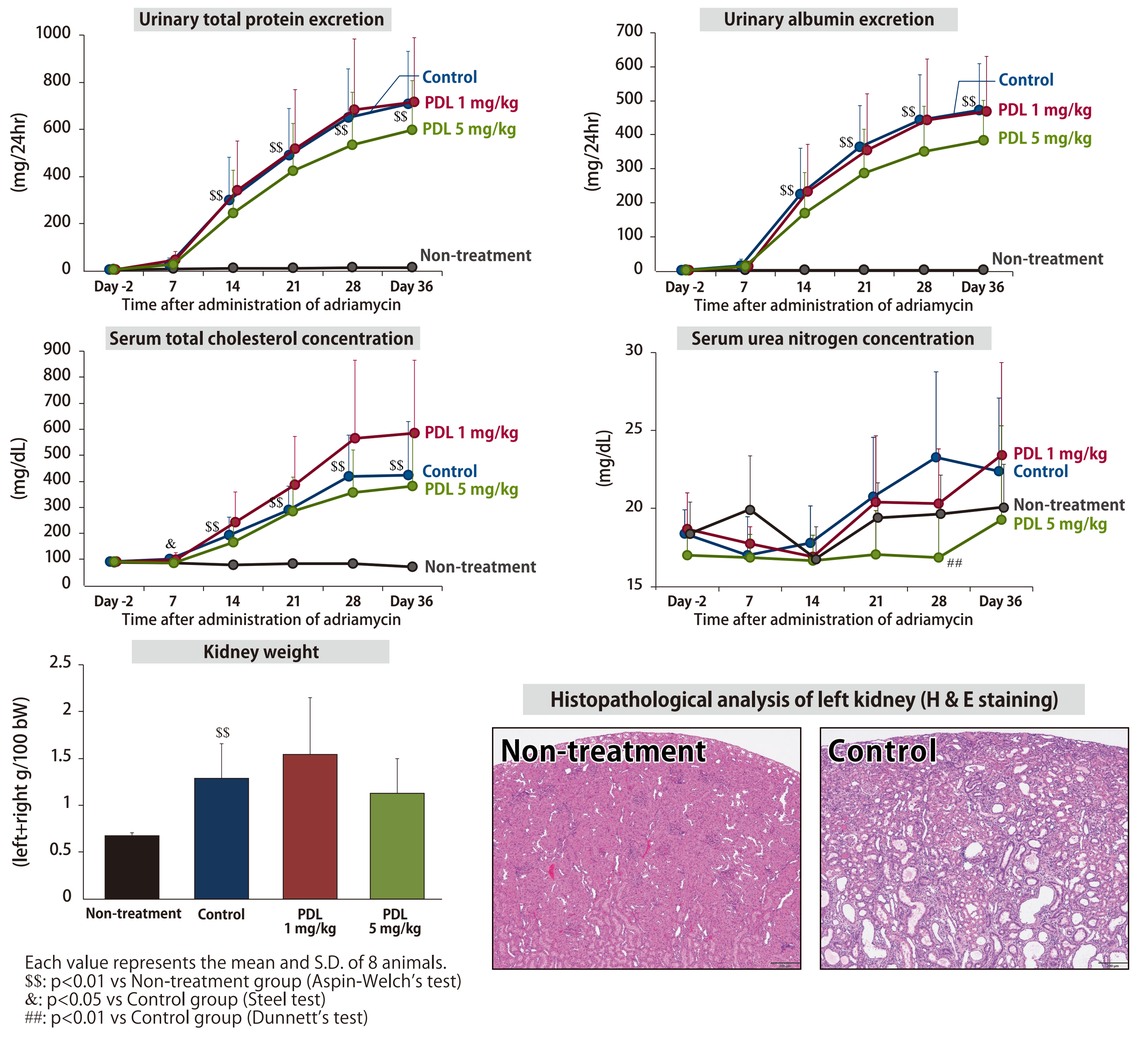
Results

Experiment 1



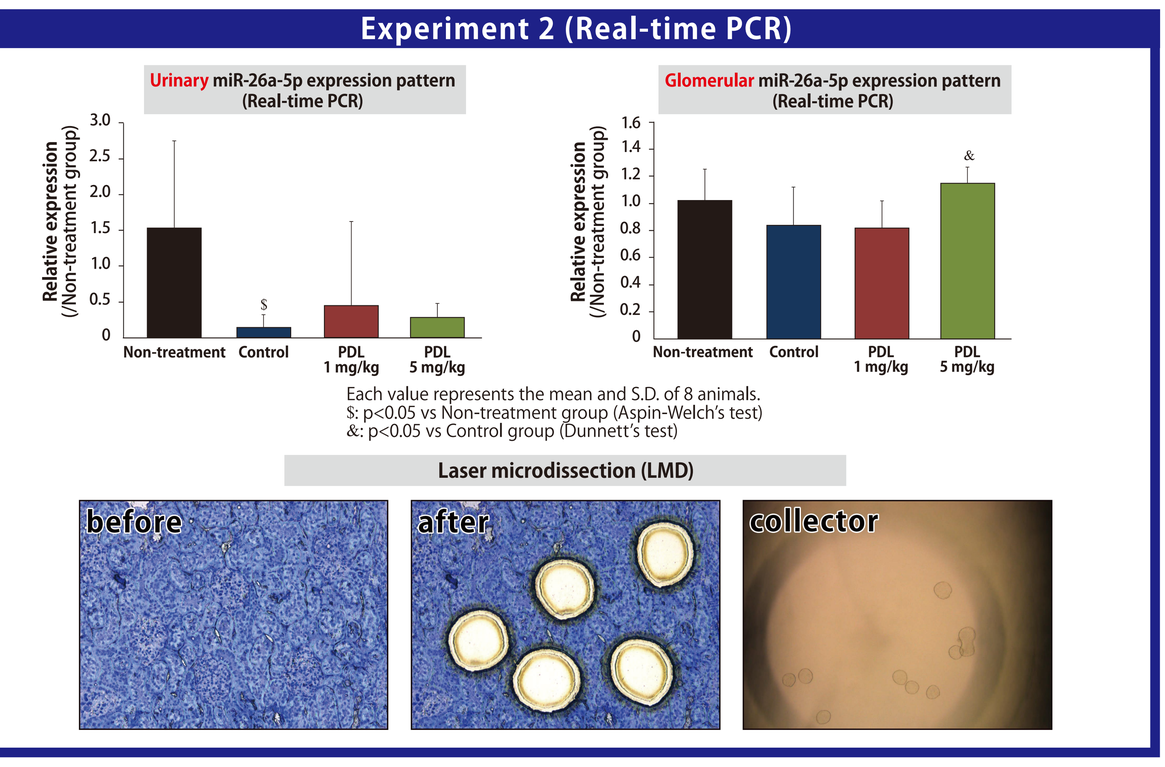
Each value represents the mean and S.D. of 7 or 8 animals.
&: p<0.05, &&: p<0.01 vs Non-treatment group (Steel test)

Experiment 2



Each value represents the mean and S.D. of 8 animals.
$$: p<0.01 vs Non-treatment group (Aspin-Welch's test)
&: p<0.05 vs Control group (Steel test)
##: p<0.01 vs Control group (Dunnett's test)

Experiment 2 (Real-time PCR)



Each value represents the mean and S.D. of 8 animals.
$: p<0.05 vs Non-treatment group (Aspin-Welch's test)
&: p<0.05 vs Control group (Dunnett's test)

Conclusion

- **Results of Experiment 1 (the percent of survival, the urinary total protein excretion and the urinary albumin excretion) indicated that ADR at 5 mg/kg was found to be suitable for induction of the nephropathy model rats.**
- **By the administration of prednisolone at 1 mg/kg, the urinary total protein excretion, the urinary albumin excretion and serum total cholesterol concentration were not improved (Experiment 2).**
- **The administration of prednisolone at 5 mg/kg was observed to be effective on dysfunction of the kidney by urinalysis and blood chemistry-analysis.**
- **As already known, the expression of miR-26a is decreased following nephropathy symptoms progression, and we analyzed the expression miR-26a in urine and the glomerulus in this study.**
- **In the ADR administration group (control group), the expression of urinary miR-26a was significantly decreased from the non-treatment group.**
- **In the PDL 5 mg/kg administration group, the expression of glomerular miR-26a was significantly increased from the control group.**
- **ADR-induced nephropathy rat model and the measurement of miR-26a are useful in evaluation of developing therapeutic drugs under the present experimental condition.**