Mediford Corporation

Effects of an immunosuppressive agent on the anti-glomerular basement membrane nephritis model rat

○ Kimihide Okamoto, Shinichi Ozato, Hideaki Kamaga, Kousuke Morizumi, Seiichi Katayama, Naoyuki Hironaka, Katsuhide Nishi

Objective

Anti-glomerular basement membrane (**GBM**) nephritis is caused by the production of autoantibody against GBM. The progression of the disease leads to kidney failure which lowers the QOL of patients considerably. Therefore, the development of novel therapeutic agents is expected.

The rat model induced by administration of anti-GBM antibody is considered as one of the experimental models of anti-GBM nephritis. In the present study, effects of cyclophosphamide (**CYP**), a typical immunosuppressive agent, were evaluated using this model.

The results indicate the animals in Nephritis model group developed anti-GBM nephritis-like symptoms, and the high dose of CYP suppressed progression of anti-GBM nephritis-like symptoms by suppressing renal glomerulus and tubules inflammation.

Summary in Japanese

抗糸球体基底膜腎炎とは，糸球体基底膜（**GBM**）に対する自己抗体の産生および沈着により誘導され，急速な腎機能の低下が特徴的な疾患である．病態の進行により引き起こされる腎不全は患者のQOLを著しく低下させるため，抗GBM腎炎に対する新規治療薬の開発が待ち望まれている．実験モデルの一つとして抗GBM抗体投与による腎炎ラットモデルが知られている．本研究では，抗GBM腎炎モデルを作製し，免疫抑制剤であるシクロホスファミド（**CYP**）の効果を検討した．

病態モデル動物は雄性のWKYラットに抗GBM抗体を単回静脈内投与することで作製した．CYPは1日1回，28日間経口投与した．評価は血漿中BUN，体重比腎臓重量，尿中タンパク排泄量，尿潜血スコア，尿中Clusterin，KIM-1，MCP-1濃度および病理組織学的検査にて行った．

病態群では正常群と比較して血漿中BUN，各種尿中パラメーター，尿潜血スコアの増加，体重比腎臓重量の高値および病理組織学的検査では半月体の形成が認められ，病態群における抗GBM腎炎様症状が観察された．この病態群の変化に対してCYP投与群は，血漿中BUNおよび各種尿中パラメーターの増加を抑制し，体重比腎臓重量で低値を示した．以上より，CYPは抗GBM腎炎モデル動物の病態進行を抑制することが示唆された．

抗GBM腎炎ラットモデルは新規治療薬の効果を評価するために有用なモデルであり，今後の治療薬開発に貢献しうると考えられる．

Materials and Methods

● Animals

Male, WKY rat, 7-week-old (on Day 0)

● Induction of anti-GBM nephritis

Antibody: Nephritogenic Monoclonal Antibody, Clone b35, Chondrex, Inc., 100 μg/1.0 mL/body

Administration: single dose via intravenous injection, Day 0

● Administration

CYP: Endoxan® Oral Powder, Shionogi & Co., Ltd., administrated orally once a day from Days 0 to 27.

● Sampling and Method

Urine collection: Housed in metabolic cage for 24 hours and urine was collected.

Plasma collection: Blood was collected from subclavian vein using syringe with heparin and centrifugated to obtain plasma.

Dissection: Measurement of kidney weight was performed.

● Evaluation

Body weight: once a week

Plasma blood urea nitrogen (BUN): 7180 Clinical Analyzer, Hitachi High-Tech Corp.

Kidney weight ratio: per 100 g body weight

Urinary protein excretion: 7180 Clinical Analyzer, Hitachi High-Tech Corp.

Score of occult hematuria: Hemastix®, Siemens Healthcare Diagnostics K.K. The scoring criteria are shown in the table below.

Urinary clusterin, KIM-1 and MCP-1: Bio-Plex Pro™ RBM Rat Kidney Toxicity Panel 1, Bio-Rad Laboratories, Inc.

Histopathological analysis:

Hematoxylin-eosin (HE) staining analysis: kidney, formalin-fixed paraffin-embedded block

Immunofluorescent (IF) staining analysis: kidney, frozen block

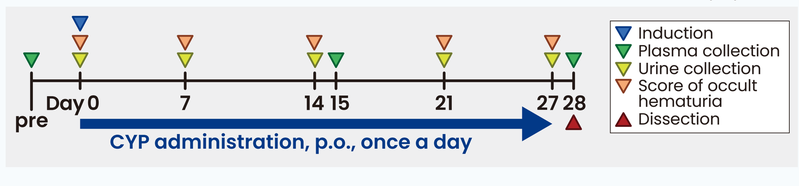
IgG: Goat Anti-Rat IgG H&L (Alexa Fluor® 488), Abcam plc.

● Experimental group, scoring criteria of occult hematuria and schedule

Group	Dosing solution	Administration	N
Normal control Nephritis model	Distilled water	p.o. 5 mL/kg	5
CYP 2 mg/kg CYP 5 mg/kg	CYP		

Score	Criteria
1	negative
2	± (non-hemolysis)
3	± (hemomlysis)
4	1+
5	2+
6	3+

The score of occult hematuria was determined based on the change in color of the test paper.



Results

Photo 1：HE (Glomerulus)

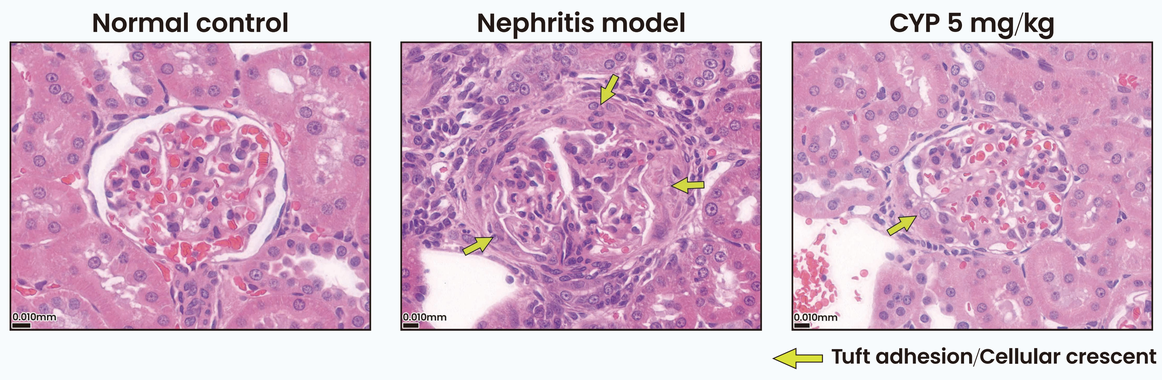


Photo 2：HE (Renal tubules)

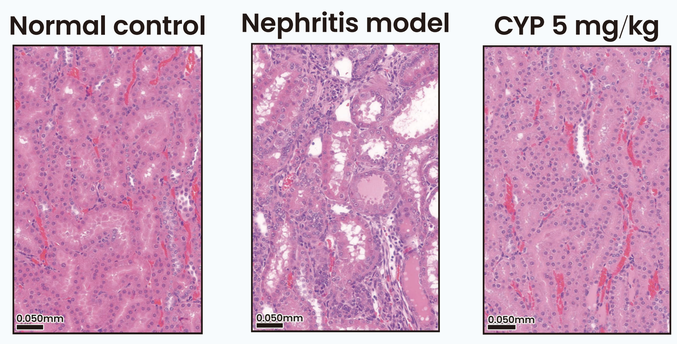


Photo 3：IF (Glomerulus)

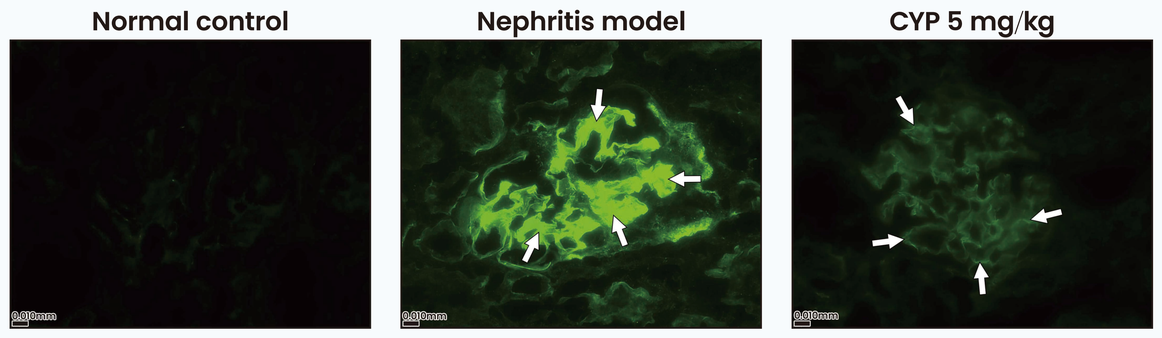


Fig. 1 Body weight

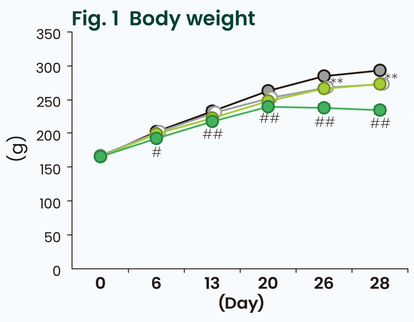


Fig. 2 Plasma BUN

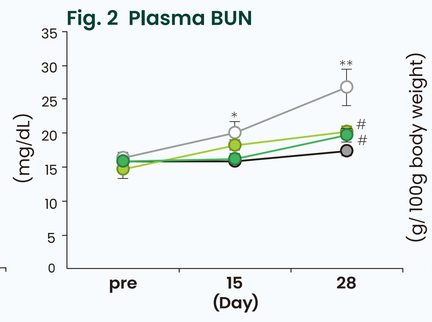


Fig. 3 Kidney weight ratio

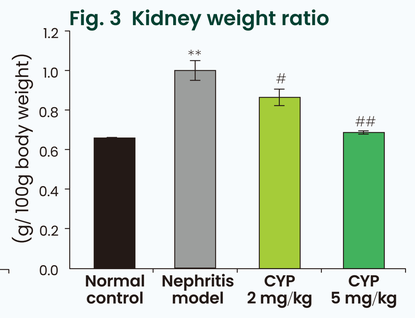


Fig. 4 Urinary protein excretion

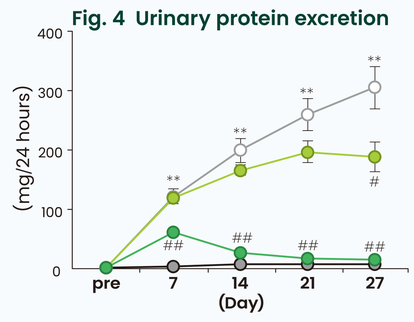
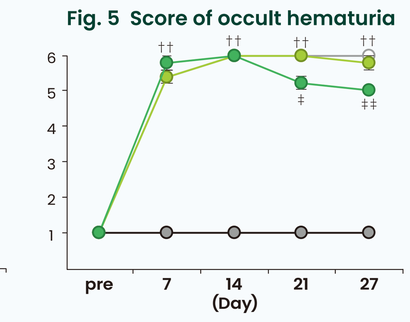


Fig. 5 Score of occult hematuria



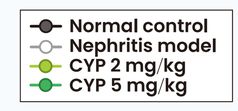


Fig. 6 Clusterin

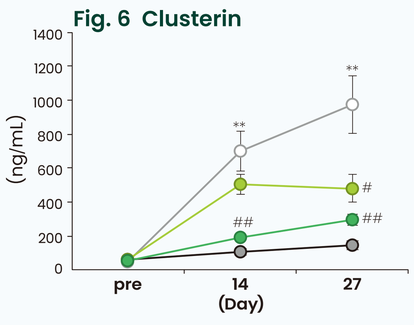


Fig. 7 KIM-1

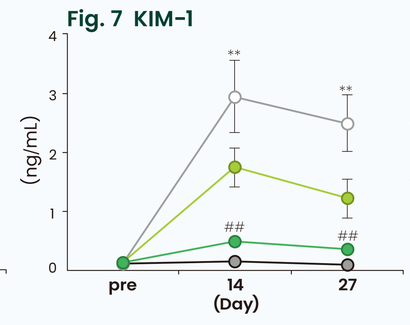
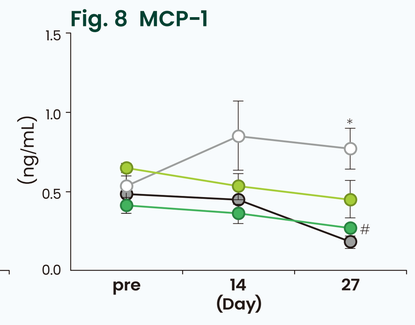


Fig. 8 MCP-1



● Statistical analysis

Fig. 1 to 4, 6 to 8:

Student's t-test: significantly different from Normal control group (*: P<0.05, **: P<0.01).
Dunnett's multiple comparison test: significantly different from Nephritis model group (#: P<0.05, ##: P<0.01).

Fig. 5: Wilcoxon signed-rank test: significantly different from Normal control group (††: P<0.01).
Steel's multiple comparison test: significantly different from Nephritis model group (‡: P<0.05, ‡‡: P<0.01).

Each symbol represents Mean ± SEM (N=3 to 5)

Conclusion

- Plasma BUN, urinary protein excretion, score of occult hematuria, urinary clusterin, KIM-1 and MCP-1 significantly increased in the anti-GBM nephritis model animals, compared with those in the normal control group (**Fig. 2 to 8**).
- The Nephritis model group showed formation of cellular crescents (**Photo 1**), endocapillary hypercellularity (**data not shown**), hyaline casts and regenerative tubules (**Photo 2**) compared with those in the normal control group.

These results indicated glomerular and tubular injury in the model animals. The tubular injury seen in the Nephritis model group would be mediated by inflammation caused by glomerular injury.

- The high dose of CYP lowered the value of plasma BUN (**Fig. 2**) and suppressed cellular crescent formation (**Photo 1**) and endocapillary hypercellularity (**data not shown**). This result indicates CYP attenuated glomerular injury via inflammation suppression.
- The high dose of CYP also lowered the values of urinary clusterin, KIM-1, MCP-1 and decreased hyaline casts and regenerative tubules (**Fig. 6 to 8 and Photo 2**). This result indicates CYP attenuated tubular injury via inflammation suppression.
- The high dose of CYP decreased urinary protein excretion from Day 7 (**Fig. 4**).

These results indicate CYP suppressed progression of anti-GBM nephritis-like symptoms by suppressing renal glomerulus and tubules inflammation.

In conclusion, this anti-GBM nephritis model is useful to evaluate the effects of the novel therapeutic agents.