# 加速型腎毒血清腎炎模型在純系小鼠的確立與澤瀉在此腎炎模型的藥效評估

# Establishment of the accelerated nephrotoxic serum nephritis in inbred mice and the effect of Alismatis Rhizoma on the the nephritis


## 中文摘要

腎毒血清腎炎是模擬人類因免疫引起所導致腎絲球腎炎的實驗模型，本研究藉投予兔子的抗基底膜抗體血清而成功地誘發於純系小鼠上。臨床上治療腎臟疾病的漢方多用於改善浮腫、尿不利、煩渴等，而澤瀉(Alismatis Rhizoma)是這類方劑主要組成之一；此生藥富含三萜類成分包括 alisol A, B 及其相關成分，由於結構相似於類固醇，而可能有類似類固醇的藥理活性，故本研究評估澤瀉在此腎炎模型的藥效。

誘發腎炎實驗以 6 週齡雌鼠(C57BL/6J mice)爲宿主，先在小鼠足底皮下注射正常兔子之 IgG；5 日後實驗組分三組分別尾靜脈注射腎毒血清 NTS (100ml, 150ml, 200ml)，對照組則給予正常兔子血清。藥效評估實驗依前述方法給予 NTS 200ml，隔天起實驗組小鼠經口投予澤瀉濃縮劑 Alisma orientale JUZEPCZUK, 澤瀉成分 alisol B acetate, 類固醇 Methylprednisolone succinate (MPS), 與澤瀉併用 MPS，一天一次連續 14 天，而對照組則給予蒸餾水。小鼠每週一次使用特製新陳代謝器收集 12 小時尿液，並在注射腎毒血清二週後用乙醚麻醉，全數犧牲以採集血清及腎臟組織。

測定尿蛋白含量與血清之尿素氮及肌胺酸酐值，來評估小鼠腎功能；其中之一枚腎組織使用 PAS 染色觀察病理組織改變，另一則製成冷凍切片以進行免疫組織化學之卵白素-生物素複合物染色，選擇一次抗體包括老鼠巨噬細胞 F4/80, CD4+與 CD8+ T 細胞，及單核球趨化蛋白一(MCP-1)等，以辨識發炎部位之特異性抗原。

實驗結果顯示，腎毒血清 200ml 實驗組之尿蛋白含量、血清之尿素氮及肌胺酸酐值皆明顯增加，且 14 天內之組織觀察即可發現腎絲球肥大與腎微血管形成血栓而閉合、新月體的形成、腎間質細胞浸潤、腎小管萎縮與輕微腎間質纖維化。在免疫組織化學染色中，腎組織有巨噬細胞、CD4+ T 細胞浸潤與 MCP-1 沈積呈色。此外，小鼠投予澤瀉濃縮劑 4mg/kg, alisol B acetate, MPS, 與澤瀉併用 MPS 等四組之尿蛋白、血清尿素氮及肌胺酸酐值顯著降低，鏡檢觀察皆有緩解病理組織惡化與減少細胞浸潤，在此腎炎的療效依序是 MPS, 澤瀉併用 MPS, alisol B acetate, 與澤瀉 4mg/kg。研究結果揭示腎毒血清腎炎是因細胞性免疫而造成腎絲球損傷損傷，期望藉由阻斷此免疫路徑來作爲未來治療腎絲球腎炎之藥物研發方向。

## 英文摘要

Nephrotoxic serum (NTS) nephritis is a well-established experimental model of human glomerular immune injury resulting in glomerulonephritis. First of two parts that the study to establish accelerated NTS nephritis that is produced in C57BL/6J mice, using anti-murine glomerular basement membrane (GBM) rabbit antiserum. The Kampo therapeutics on renal disease to ameliorate symptoms such as edema, oliguria and thirst, and the Alismatis Rhizoma is the major component of these prescriptions. The herb contains active triterpenoids including Alisol A, B and their related compounds, have been reported regarding their therapeutic activities similar to steroids. Second of two parts that the study to evaluate the effect of Alismatis Rhizoma on the nephritis.

In the first experiment, C57BL/6J mice (6weeks, females) was pre-immunized with the normal rabbit IgG in rear footpads. Five days later, mice were received intravenously injection with 100, 150 and 200ml of NTS, respectively. Control mice were treated for normal rabbit serum. In the second experiment, a similar protocol was followed, but treatment with 200ml of NTS. One day later, the experimental groups were administered orally the Alisma orientale JUZEPCZUK (AO), alisol B acetate, Methylprednisolone succinate (MPS) and AO combine with MPS once daily for 14 days. The control group was untreated by the same method. The urine of experimental animals was collected using metabolic cages once a week for 12h. The mice were sacrificed under ether narcotization 14 days after the injection of NTS. Sera and kidney tissues were obtained at sacrifice.

The urinary protein content, blood urea nitrogen (BUN) and serum creatinine (SCr) were determined. Renal tissues were served to histological examination (PAS stain), the glomerular changes and tubulo-interstitial (TI) changes were evaluated (0 to 4+) separately. The frozen sections used the Avidin-biotin-peroxidase complex (ABC) technique as immunohisto- chemistry stain. Choice the antibodies include murine F4/80 macrophage, CD4+ T cell, CD8+ T cell, MCP-1 (monocyte chemotactic protein-1) to recognize the specific antigens, which deposited in inflammatory site. The result of the first experiment was revealed the urinary protein, BUN and serum creatinine in the NTS 200ml group were significant increased. The histological examination was observed the typical NTS nephritis with cellular proliferation in glomeruli, occlusion of glomerular loops, crescents and tubulointerstitial changes within 14 days. In the ABC stain assay, cells that T-cell such as macrophage, CD4+ T cell and chemokine which MCP-1 were localized in the renal tissues. In the second experiment, the urinary protein, BUN and SCr in the experimental groups which treatment AO extract 4mg/kg, alisol B acetate, Methylprednisolone succinate (MPS) and AO combine with MPS were significant decreased versus NTS control group. On

the other hand, the histological examination and the ABC stain assay were observed the alleviation in the therapeutic groups. According to the following sequence, MPS was the priority effect, than AO combine with MPS, alisol B acetate, AO extract 4mg/kg. The results were revealed the immune response of the NTS nephritis would be generates by cell-mediated immunity injury and drug development by blocking the immunomediative pathway in the future.