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論　　文　　名	「Toxicological Studies on an Antineoplastic Platinum Complex Nedaplatin in Rats（ラットにおける抗腫瘍性白金錯体ネダプラチンの毒性学的研究)」
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論文要旨

Preface

Cancer is a leading cause of death worldwide. Treatments aimed at curing, prolonging life and improving the quality of life of patients with cancer, as well as supportive care, are ways in which the burden of cancer can be reduced. In treatment, chemotherapies have been the mainstay for almost 100 years, helping improve the survival rate of patients significantly. Therefore, the pharmaceutical industry has a mission to discover and develop new anticancer drugs through innovative discovery technologies and development approaches.

Antineoplastic platinum complexes have proven to be effective and are widely applied to several human malignancies. Among of them, cisplatin (CDDP) is one of the most widely used antineoplastic agents. However, its clinical use is limited by its potent nephrotoxicity, which can lead to acute renal failure. On the other hand, nedaplatin (NDP) is a novel antineoplastic platinum complex with reduced nephrotoxicity. It causes myelosuppression, particularly resulting in thrombocytopenia,

as the dose-limiting toxicity, but nephrotoxicity occurs less frequently. However, detailed toxicological data concerning NDP in the experimental model are still limited, and a more complete evaluation of its nephrotoxic effects should be necessary.

A series of this study were conducted firstly 1) to elucidate the functional and morphological changes in the kidney of rats treated with NDP by comparing those in CDDP-treated rats (Chapter 1), and secondarily 2) to investigate the ameliorative effects on the nephrotoxicity following NDP treatment by the modification of dosing protocol and to clarify the mechanism behind the effect (Chapter 2). Recently, toxicogenomics has been expected to accelerate drug development and aid risk assessment. Therefore, finally 3) the gene expression profiles were analyzed in the kidney (particularly, renal papilla) following NDP treatment in rats using DNA microarrays to elucidate the molecular mechanism of NDP-induced nephrotoxicity and to identify potential biomarker genes (Chapter 3).

Chapter 1 Toxicological Characterization of a Novel Antineoplastic Platinum Complex Nedaplatin (NDP)

Section 1.1 A Comparative Study on Nephrotoxicity of Cisplatin (CDDP) and Nedaplatin (NDP) in Rats

The present study was designed to elucidate the functional and morphological changes in the kidney of rats treated with NDP by comparing those in CDDP-treated rats. A single bolus dose of 15 mg/kg NDP or 7.5 mg/kg CDDP was injected intravenously to 8-, 11-, or 15-week-old male and female rats, which were then sacrificed after 10 days. As a result, CDDP treatment markedly increased urinary excretion of protein and marker enzymes; NDP also increased them, but to a lesser extent. CDDP increased plasma creatinine and blood urea nitrogen in rats of all age groups at necropsy. No apparent changes were seen following NDP treatment except in the 15-week-old rats. These results suggested that NDP is less nephrotoxic than CDDP. CDDP-treated rats showed remarkable proximal tubular lesions in the renal cortex and corticomedullary region, and the papillary lesions were minor. On the other hand, the NDP-induced nephrotoxicity was morphologically characterized by hyaline droplet changes, necrosis or hyperplasia of the collecting duct epithelium in the renal papilla and the epithelium covering the papilla. In short, NDP is a promising second-generation platinum complex with reduced nephrotoxicity.

Section 1.2 Time Course of the Change of Nedaplatin (NDP)-Induced Nephrotoxicity in Rats

The present experiment was conducted to characterize the time course of changes of NDP-induced nephrotoxicity in rats. A single bolus dose of 6 or 12 mg/kg NDP was injected intravenously to rats, which were then sacrificed 2, 4, 7 and 14 days after dosing. NDP-induced

nephrotoxicity was initially characterized by single cell and/or focal necrosis in the epithelium of distal tubules and collecting ducts as well as proximal tubules. In the later stage, subsequent cystic dilatation and regeneration occurred in these affected tubules, but incomplete tissue repair was still observed in the kidney 14 days after dosing. In short, the present study has demonstrated that the characteristic time course of changes of the nephrotoxicity associated with the NDP treatment. Moreover, it was suggested that attention must be paid to the nephrotoxicity as well as myelotoxicity during the NDP treatment.

Chapter 2 Amelioration of Nedaplatin (NDP)-Induced Nephrotoxicity

Section 2.1 Ameliorative Effect by Hydration on Nedaplatin (NDP)-Induced Nephrotoxicity in Rats

The present experiment was conducted to evaluate whether hydration is useful for the amelioration of the nephrotoxicity. Nonhydrated (Nhyd) or hydrated (Hyd) rats, treated with a single intravenous dose of 20 mg/kg NDP, were sacrificed 7 days after dosing. As a result, NDP-induced nephrotoxicity was dramatically reduced by hydration, while it had no clear effects on myelotoxicity. Measurement of urinary platinum excretion revealed that total amount of platinum excretion was significantly higher in Hyd-NDP rats than that in Nhyd-NDP rats. In terms of its urinary concentration, Hyd-NDP rats showed lower concentration compared to that in Nhyd-NDP rats. In short, pre- and post-hydration at dosing is effective at minimizing the nephrotoxicity of NDP.

Section 2.2 Ameliorative Effect by Continuous Infusion on Nedaplatin (NDP)-Induced Nephrotoxicity in Rats

The current experiment was conducted to evaluate whether prolongation of infusion time is useful for the amelioration of NDP-induced nephrotoxicity. Rats were treated with 12 mg/kg NDP with the following dosing protocols, bolus injection, 1- or 4-hour continuous infusions, and sacrificed 3 days after dosing. NDP-induced nephrotoxicity was dramatically reduced by the prolongation of infusion time, while it had no clear effects on myelotoxicity. In short, prolongation of the infusion time is effective at minimizing the nephrotoxicity of NDP.

Chapter 3 Comparative Analysis of Gene Expression between Renal Cortex and Papilla in Nedaplatin (NDP)-Induced Nephrotoxicity in Rats

To elucidate the molecular mechanism of NDP-induced nephrotoxicity and to identify potential biomarker genes with a particular focus on the renal papillary toxicity, the gene expression profiles were analyzed in two renal regions, the cortex and the papilla, following NDP treatment in rats using DNA microarrays. Rats received a single intravenous dose of 10 mg/kg NDP or vehicle alone (5% xylitol solution) were sacrificed 6 days later. Global gene expression analysis revealed

that several genes involved in various functional categories were commonly deregulated in both renal regions, such as apoptosis, cell cycle regulation, DNA metabolism, cell migration/adhesion and cytoskeleton organization, or genes induced as a perturbation of oxidative status and calcium homeostasis. Comparative analysis of gene expression between the renal cortex and papilla revealed that genes encoding several subtypes of cytokeratins were identified as being specifically overexpressed in the papilla by the NDP treatment. Immunohistochemistry confirmed increased expression of cytokeratins 14 and 19 at the epithelium covering the papilla and/or the collecting duct epithelium. Overall, the present results contribute to understanding the renal molecular events of NDP-induced nephrotoxicity including novel potential biomarker genes that may serve as indicators of renal papillary toxicity.

Conclusions

1. NDP is a promising second-generation platinum complex with reduced nephrotoxicity. However, it was suggested that attention must be paid to the nephrotoxicity as well as dose-limiting myelotoxicity during the NDP treatment.

2. NDP-induced nephrotoxicity was morphologically characterized by single cell and/or focal necrosis followed by cystic dilatation and regeneration not only in the proximal tubule but also in the distal tubule and the collecting duct.

3. The hydration was effective at minimizing the nephrotoxicity of NDP. The amelioration effect could be interpreted as resulting from hydration causing polyuria and diluting the platinum concentration in the urine and also shortening the platinum contact time with the renal tubules.

4. The prolongation of the infusion time was effective at minimizing the nephrotoxicity of NDP. The amelioration effect could be interpreted as resulting from prevention of temporary elevation of urine platinum concentration.

5. Mechanistically, the gene expression pattern elicited by NDP treatment suggested the occurrence of apoptosis and the perturbation of oxidative status. Among the several genes specifically reflected the renal papillary damage, cytokeratins 14 and 19 were one of the most promising biomarker candidates, the increased expression of which on the protein levels were confirmed by immunohistochemistry.

6. The animal model used in these studies is likely to be useful for analyzing pathophysiological events of acute renal failure following the treatment of antineoplastic platinum complex.

審査結果の要旨

癌は現代社会における最重要疾患であり，その克服は重大な課題の一つである．治療法の発展により，多くの癌患者が救命されるようになったものの，延命だけでなく社会復帰の可能な QOL の高い化学療法の開発が望まれている．

白金錯体である cisplatin（CDDP）は強力な制癌剤として繁用されているが，腎毒性が強く本剤の使用の妨げとなっている．一方，nedaplatin（NDP）は，腎毒性の軽減を目指して開発された新規の抗腫瘍性白金錯体であり，臨床的に高い有効性が確認されている．しかし，その腎毒性に関する情報は少なく，毒性学的特徴について明らかにされていない．そこで，本研究では，NDP 誘発腎毒性の毒性学的特徴を明らかにするとともに，腎毒性の軽減効果を検証した．成績の概要は以下のとおりである．

第 1 章第 1 節では，8，11 及び 15 週齢の雄ラットに NDP（15 mg/kg 体重）あるいは CDDP（7.5 mg/kg 体重）を単回静脈内投与し，CDDP との比較により NDP の腎毒性を検討した．その結果，腎機能パラメータの変動は CDDP より NDP で顕著に軽度であった．投与 10 日後の CDDP 誘発腎病変は，腎皮質あるいは皮髄境界部における近位尿細管に主座し，上皮の壊死や再生を主体としたのに対し、NDP 誘発腎病変は腎乳頭に主座し，近位尿細管の病変の程度は軽度であった．以上の結果から，NDP は CDDP と比較して腎毒性が軽減された薬物であることが示された．

第 1 章第 2 節では，8 週齢の雄ラットに，NDP（6 及び 9 mg/kg 体重）を単回静脈内投与し，腎毒性及び骨髄毒性病変の経時的変化を病理組織学的に検討した．その結果，投与後 4 日をピークに腎皮質における近位尿細管上皮の巣状壊死や単細胞壊死，集合管や腎乳頭上皮の単細胞壊死が発現した． 4 日以降，尿細管及び集合管上皮の再生がみられ始め，14 日では線維化がみられた．骨髄においては，投与後 7 日をピークに造血細胞が減少し，14 日では回復傾向を示した．以上の結果から，NDP 投与による骨髄毒性は休薬により回復を示したが, 腎毒性は不可逆的障害を残す可能性があるため, 臨床での NDP の使用においては, 腎毒性に注意を要することが示唆された．

第 2 章第 1 節では，8 週齢の雄ラットに NDP（20 mg/kg 体重）を単回静脈内投与し，投与前後の水分補給による腎毒性の軽減効果を検討した．その結果，腎毒性は投与前後の水分補給により顕著に軽減されたが，骨髄毒性には影響がなかった．以上の結果から，NDP 投与前後の水分補給により尿中の白金濃度の上昇を抑えることが，NDP の腎毒性軽減に有効であることが示された．

第 2 章第 2 節では，8 週齢の雄ラットに NDP（12 mg/kg 体重）を単回急速あるいは持続静脈内投与し，投与時間の延長による腎毒性の軽減効果を検討した．その結果，腎毒性は投与時間の延長により顕著に軽減されたが，骨髄毒性には影響がなかった．以上の結果から，NDP 投与時間の延長により血中濃度の一過性の上昇を抑えることが NDP の腎毒性軽減に有効であることが示された．

第 3 章では，8 週齢の雄ラットに NDP（10 mg/kg 体重）を単回静脈内投与し，投与後 6 日に採材した腎（皮質及び乳頭部）について，網羅的遺伝子発現解析を実施した．その結果，腎の両部位において酸化ストレス応答やアポトーシス，細胞周期調節，DNA 代謝，細胞遊走・接着分子，細胞骨格に関連する多数の遺伝子の発現に変動が認められ，NDP の腎毒性発現には，腎尿細管及び集合管への白金錯体の曝露による細胞への酸化ストレスとそれに伴う細胞死が密接に関連しているものと推察された．また，両部位間での遺伝子発現プロファイルの比較解析により，種々のサブタイプの keratin 遺伝子が腎乳頭部に特異的に発現上昇することを見出した．更に，免疫組織化学染色により NDP の腎乳頭病変発現部位に一致して，cytokeratin 14 及び 19 がタンパクレベルで発現誘導することを確認した．これらの遺伝子は，NDP 投与による腎乳頭毒性を評価・予測する上で有用なマーカー候補遺伝子となることが示唆された．

以上のように本研究は，新規抗腫瘍性白金錯体である NDP の腎毒性について，臨床応用に有用な示唆を与える幾つかの知見を見出した．また，トキシコゲノミクス研究により腎毒性の発現メカニズムを分子レベルで明らかにし，腎乳頭毒性を評価・予測する上で有用な新規のマーカー候補遺伝子を発見した．これらの成果は，医学・獣医学の発展，とりわけ毒性学・毒性病理学の新たな展開に貢献するものであり，本論文の審査ならびに学力確認の結果と併せて，博士（獣医学）の学位を授与することを適当と認める．