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# Pharmacological activation of NQO1 increases $NAD^+$ levels and attenuates cisplatin-mediated acute kidney injury in mice

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**Cisplatin is a widely used chemotherapeutic agent for the treatment of various tumors. In addition to its antitumor activity, cisplatin affects normal cells and may induce adverse effects, such as ototoxicity, nephrotoxicity, and neuropathy. Various mechanisms, such as DNA adduct formation, mitochondrial dysfunction, oxidative stress, and inflammatory responses, are critically involved in cisplatin-induced adverse effects. As $NAD^+$ is a cofactor for various enzymes associated with cellular homeostasis, we studied the effects of increased $NAD^+$ levels by means of NAD(P)H:quinone oxidoreductase 1 (NQO1) activation using a known pharmacological activator (β-lapachone) in wild-type and $NQO1^{-/-}$ mice on cisplatin-induced renal dysfunction *in vivo*. The intracellular $NAD^+$/NADH ratio in renal tissues was significantly increased in wild-type mice co-treated with cisplatin and β-lapachone compared with the ratio in mice treated with cisplatin alone. Inflammatory cytokines and biochemical markers for renal damage were significantly attenuated by β-lapachone co-treatment compared with those in the cisplatin alone group. Notably, the protective effects of β-lapachone in wild-type mice were completely abrogated in $NQO1^{-/-}$ mice. Moreover, β-lapachone enhanced the tumoricidal action of cisplatin in a xenograft tumor model. Thus, intracellular regulation of $NAD^+$ levels through NQO1 activation might be a promising therapeutic target for the protection of cisplatin-induced acute kidney injury.**

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*cis*-Diamminedichloroplatinum II (CDDP, cisplatin) is a widely used chemotherapeutic drug for the treatment of various solid tumors in the head and neck, bladder, lung, ovaries, testicles, and uterus.[1–6] However, the use of cisplatin is limited owing to its various adverse effects, including ototoxicity, nephrotoxicity, and peripheral neuropathy, during the course of chemotherapy. Approximately one out of three patients experience a significant reduction in renal function following cisplatin treatment.[7,8] In addition to DNA damage, a positive feedback loop between inflammatory cytokines and oxidative stress causing tubular toxicity and vascular injury has been reported as the major cause of cisplatin-induced renal dysfunctions.[9–13] Post-translational modification of nuclear factor (NF)-κB p65 and p53, including phosphorylation and acetylation, may be an important factor in cisplatin-mediated cytotoxicity, because activation of these molecules has been linked to both inflammatory responses and apoptosis.[14–16] Furthermore, oxidative stress, particularly hydroxyl radical, has a major role in cisplatin-induced p53 activation through DNA damage.[6] In particular, activation of p53 by its acetylation was also critically involved in cisplatin-induced renal injury.[17]

NADH: quinone oxidoreductase 1 (NQO1) is a cytosolic antioxidant flavoprotein that catalyzes the reduction of quinones to hydroquinones by utilizing NADH as an electron donor, which consequently increases intracellular $NAD^+$ levels.[18,19] In addition, there is evidence that NQO1 has a role in other biological activities, including anti-inflammatory processes, the scavenging of superoxide anion radicals, and the stabilization of p53 and other tumor-suppressor proteins.[20–26] Several activators of the NQO1 enzyme have been identified, of which β-lapachone (3,4-dihydro-2,2-dimethyl-2*H*-naphto[1,2-b]pyran-5,6-dione; βL) is the best known.[27,28] βL was first isolated from the bark of the lapacho tree and reported to inhibit tumor growth.[29] However, recent reports indicate that the enzymatic activation of NQO1 by βL

has beneficial effects on several characteristics of metabolic syndromes, for example, prevention of health decline in aged mice, amelioration of obesity or hypertension, prevention of arterial restenosis, and protection against salt-induced renal injury.[30–35] Consistent with these reports, cellular $NAD^+$ and NADH levels have been shown to be important mediators of energy metabolism and cellular homeostasis.[36–39] As $NAD^+$ acts as a cofactor for various enzymes, including sirtuins (Sirts), poly(ADP-ribose) transferases, and cyclic ADP–ribose synthases,[40–43] the regulation of $NAD^+$ may have therapeutic benefits through its effect on $NAD^+$-dependent enzymes. In particular, several Sirt proteins are $NAD^+$-dependent protein deacetylases that have been reported to be anti-aging molecules associated with calorie restriction.[44,45] In mammals, there are seven homologs of Sir2 (Sirt1–7) that show differential subcellular localizations.[38,45] Among these, nuclear-localized Sirt1 is activated under energy stress conditions, such as fasting, exercise, or low glucose availability.[46] Sirt1 has a key role in metabolism, development, stress response, neurogenesis, hormone responses, and apoptosis[47,48] by deacetylation of substrates, such as NF-κB p65, FOXO, p53, and histones.[49–52] In addition, recent studies suggest that Sirt1 regulates inflammatory responses through NF-κB p65 deacetylation. In the absence of Sirt1 *in vivo* (knockout mice), there is deregulated inflammatory pathway activation in conjunction with increased inflammatory gene expression.[53]

Mitochondria-localized Sirt3 regulates adaptive thermogenesis, mitochondrial function, energy homeostasis, and cellular survival upon genotoxic stress.[54–56] Sirt3 exerts antioxidative effects through the deacetylation and activation of mitochondrial isocitrate dehydrogenase 2 (IDH2) and the enhancement of the glutathione antioxidant defense system. Furthermore, Sirt3 antagonizes p53 function through direct interaction and subsequent deacetylation of p53 in the mitochondria.[57]

Although a link between $NAD^+$-dependent molecular events and cellular metabolism is evident, it remains unclear whether modulation of $NAD^+$ levels has an impact on cisplatin-induced renal injury. In this study, we investigated the protective effects of βL on cisplatin-induced acute kidney injury in wild-type (WT) compared with NQO1 knockout ($NQO1^{-/-}$) mice. We found that βL protects against cisplatin-induced renal dysfunction and that this effect is mediated by Sirt1 and Sirt3 through NQO1 activation.

## RESULTS

### βL activates NQO1 enzyme activity and increases the intracellular ratio of $NAD^+$ to NADH in mice

Kidney homogenates from WT mice were isolated and treated with βL to measure NQO1 activity. As shown in Supplementary Figure S1A online, NQO1 activity was significantly increased by βL treatment (26.3 ± 2.1 vs. 11.3 ± 1.2 nmol/min/mg protein (control)), whereas it was attenuated to the control level by the addition of the NQO1 inhibitor dicumarol (14.5 ± 1.5 nmol/min/mg protein). By contrast, dicumarol itself completely abrogated NQO1 activity (1.5 ± 1.0 nmol/min/mg protein). Next, we asked whether NQO1 activation correlates with intracellular $NAD^+$ and NADH levels in kidney tissues. WT mice were orally administered βL or vehicle for 4 days, and $NAD^+$/NADH ratios were determined from isolated kidney tissues. We found a significant increase in the $NAD^+$/NADH ratio in βL-treated mice compared with the ratio in control mice (2.13 ± 0.42 vs.1.22 ± 0.3) (Supplementary Figure S1B online).

### βL inhibits cisplatin-induced acute kidney injury in mice

C57BL/6 mice were treated with βL, cisplatin, or βL + cisplatin, as indicated in Supplementary Figure S2 online, and the levels of serum creatinine and blood urea nitrogen (BUN) (biochemical markers for kidney dysfunction) were measured at day 4. As shown in Figure 1a and b, cisplatin increased the levels of serum creatinine and BUN (1.67 ± 0.12 and 126 ± 7.5 mg/dl, respectively), compared with control (0.31 ± 0.11 and 36.0 ± 7.4 mg/dl, respectively). However, βL + cisplatin significantly reduced the levels of both serum creatinine (1.01 ± 0.15 mg/dl) and BUN (79.8 ± 4.1 mg/dl), as

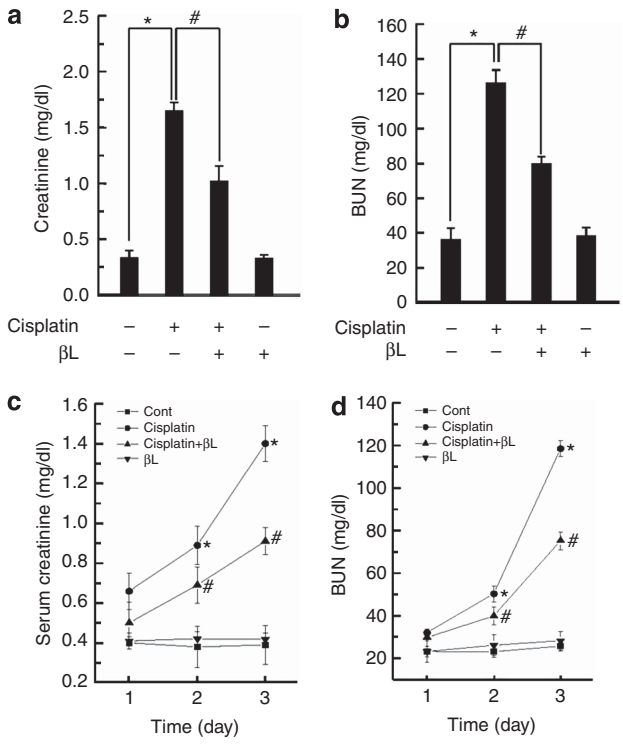


**Figure 1 | Effect of β-lapachone (βL) on serum creatinine and blood urea nitrogen (BUN) in cisplatin-induced acute kidney injury.** βL (40 mg/kg body weight) was administered orally once a day for 4 consecutive days. Cisplatin (20 mg/kg body weight) was injected once at 12 h after the first βL administration. The mice were killed at 72 h after the single cisplatin injection, and levels of (**a**) serum creatinine and (**b**) BUN were analyzed using an assay kit according to the manufacturer's instructions (BioVision). To observe the effect of βL on the cisplatin-induced toxicity with the experimental time course, the mice were killed daily after cisplatin injection, and serum was analyzed for (**c**) creatinine and (**d**) BUN. $^{*,\#}P < 0.05$ by one-way analysis of variance compared with the control (*) and cisplatin + βL group (#) ($n = 5$).