P1-1

Dietary nitrite is an alternative source of NO in vivo

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Purpose: Nitrite and nitrate in the blood are recognized as a waste form of nitric oxide (NO). Recently, we demonstrated that pharmacological dose of oral nitrite increases circulating NO and NO is generated from nitrite during renal ischemia. In this study, we examined the effect of chronic administration of dietary dose of nitrite on renal injury induced by chronic L-NAME intake in rats.

Material and methods: We prepared nitrite-containing drinking water (0.1, 1.0, 10, and 100 mg $NaNO_2$/liter, which corresponds to 0.003, 0.03, 0.3, and 3 mg $NaNO_2$/day/rat) and administered to L-NAME-treated hypertensive rats for eight weeks. We evaluated the blood NO levels as a hemoglobin-NO adducts (Hb-NO) using electron paramagnetic resonance (EPR) method. Blood pressure was measured by tail-cuff method.

Results: Treatment with L-NAME (1g/liter of drinking water) alone significantly reduced the blood HbNO-derived EPR signal ($p<0.01$), and nitrite treatment restored the L-NAME-induced HbNO signal reduction, but it did not exceed control level of HbNO even high dose of nitrite treatment (100 mg $NaNO_2$/liter). In addition, co-administration of nitrite improved the morphology change of glomerulus.

Conclusion: Our present results suggesting that oral ingestion of nitrite can be, in part, an alternative to L-arginine as a source of NO.

P1-2

Proteomic Analysis of S-Nitrosation in Spinach Chloroplasts Much More Beyond S-Nitrosated Target Screening

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Purpose: Nitric oxide is an important signal molecule which may convey its bioactivity in significant part through S-nitrosation, however, the substrate specificity of this post-translational modification remains unclear.

Material and methods: We zoomed this issue in spinach chloroplasts with biotin-switch assay coupled with two-dimensional PAGE protein separation followed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry analysis.

Results: The protein profiles of the total proteins, the free thiol-containing proteins, and the S-nitrosated proteins were compared and we found that almost all the thiol-containing proteins were S-nitrosated. Therefore, we proposed an open question "how specific the S-nitrosation could be" beyond the analysis of the S-nitrosated proteins. In addition, the modification of the biotin-switch method to improve the yield of S-nitrosated proteins, the analysis of the sensitivity of biotin-switch method, and the simple thiol-containing protein purification assay presenting here are also very helpful for the future study.

Conclusion: Our results remind researchers to present the total protein profile and the thiol-containing protein profile as control when using proteomic approach in the study of S-nitrosated proteins in order to get the real specific S-nitrosated proteins involved in the process, otherwise the screened potential targets are ambiguously specific.

P1-3

Nitric oxide from inducible nitric-oxide synthase does not exacerbate adriamycin -induced tubulointerstitial injury.

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Purpose: It is well known that oxidative stress is related to the pathogenesis of adriamycin (ADR) nephropathy. But it is unclear how nitric oxide (NO) is associated with the pathological process after ADR administration.

Material and methods: To study the role of NO after ADR administration, at first, we measured urinary excretion of NOx (NO2 + NO3), stable metabolites of NO, on day 1. Next, NO in the kidney homogenate was assayed by electron paramagnetic resonance spectroscopy using direct NO trapping technique 4, 6, 8, 12, 24 h after ADR administration and 6 h after ADR administration treated with 1400W as a specific inducible nitric oxide synthase (iNOS) inhibitor. Urinary excretion of 8-hydroxydeoxyguanosine (8OHdG) and N-acetyl-beta-D-glucosaminidase (NAG) were measured as a marker of oxidative stress and tubulointerstitial injury, respectively.

Results: The levels of NO after ADR administration gradually elevated up to 6 h and became statistically different compared to that in controls. Pretreatment with 1400W attenuate the increase of NO level without normalizing the NAG level. In contrast, urinary excretion of 8OHdG after ADR administration was significantly higher than that of controls on day 1.

Conclusion: These findings suggest that iNOS derived NO did not show a harmful effect on tubulointerstitial damage in ADR nephropathy.

P1-4

Pivotal role of trans-arachidonic acids in microvascular tone and survival: Novel mediators of nitrative stress

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Purpose: Nitrative stress plays an important role in microvascular degeneration leading to cerebral ischemia. Trans-arachidonic acids (TAAs) are generated endogenously via the NO2•-mediated isomerization of arachidonic acid. We have recently described that TAAs induce a retinal microvascular injury. We hypothesized that TAAs could be important mediators in the regulation of cerebral blood flow and induction of cerebral microvascular lesions.

Material and methods: We examined the effect of TAAs in microvascular survival. We generated a unilateral cerebral hypoxic-ischemic animal model of cerebral ischemia on newborn pups to measure TAA levels produced in vivo in the brain. The cytotoxic effect of TAAs was assessed in vivo by injection of these products in lateral ventricles and ex vivo by treatment of brain explants. The role of the anti-angiogenic factor TSP-1 was determined.

Results: Brain TAA levels increased markedly 18 h post-hypoxia-ischemia reaching micromolar concentrations. Physiological concentrations of TAAs mediated an endothelium- and guanylyl cyclase-dependent vasodilation. However, longer exposure caused microvascular degeneration in vitro, in vivo and ex vivo. These effects were mediated by an upregulation of the anti-angiogenic factor TSP-1.

Conclusion: Our findings provide new insights into the molecular mechanisms of nitrative stress on microvascular injury and suggest a pivotal role for trans-arachidonic acids in ischemic encephalopathies.