Introduction: Oxidative and nitrosative stresses are important for mediating secondary damage following spinal cord injury (SCI). A key player in this process is likely to be nitric oxide (NO) because of its various physiological and pathological roles in the CNS. Arginase, a major metabolic enzyme of the liver, is involved in the regulation of NO production. It does this by competing with nitric oxide synthase (NOS) for the same substrate, L-arginine. However, the exact pathway by which arginase is linked to oxidative-nitrosative stress during SCI is unclear. For the successful reconstruction of spinal cord defects using biomaterials, it is important to first elucidate the mechanism of cellular damage following spinal cord transection. The aim of the present study was therefore to investigate NO, nNOS and endogenous arginase for possible roles in the mechanism of tissue damage following spinal cord transection injury (SCTI) in a rat model.

Materials and Methods: Experimental groups and surgical procedures

Adult female rats were randomly assigned to 3 groups: the sham operated control group (Th9 laminectomy only), the vehicle group (SCTI + saline controls) and the treatment group (SCTI + arginase inhibitor Nω-Hydroxy-nor-L-arginine at 2.5 mg/kg/day for 3 days). Three days after the operation, a 1 cm length spinal cord segment containing the injury epicenter was removed.

NOx measurement

Generation of NO was expressed as NOx, nitrite (NO2-) and nitrate (NO3-). Nitrate reductase and NADPH were added to convert NO3- to NO2-. An NO analyzer (280i, Sievers, Boulder, CO) was then used to quantify NO2-.

Western blot analysis

Each sample was separated by SDS/PAGE and then transferred onto PVDF membranes. The membranes were incubated with the following primary antibodies: polyclonal rabbit anti-nNOS, monoclonal mouse anti-nitrotyrosine and polyclonal rabbit anti-arginase.

Arginase activity assay

10mM MnCl2 was incubated with tissue homogenate for 10min. The mixture was then incubated in the presence of 0.5M arginine, pH9.7 for 1 hour. 9% isonitrosopropiopheanone was added and heating for 45min. The amount of urea formed was determined spectrophotometrically at 540 nm.

TUNEL staining

Frozen sections taken from a position 1 mm rostral to the injury site were incubated with an In Situ Apoptosis Detection Kit as per the manufacturer’s instructions (TaKaRa, Japan) before counterstaining with hematoxylin.

Results: In the vehicle group, significant increases were observed in arginase activity and protein expression, nitrotyrosine content and the percentage of TUNEL positive cells compared to the sham operated control group. In contrast, the expression of nNOS protein decreased while NO production was unchanged. In the treatment group, the increases in nitrotyrosine content and TUNEL positive cells were lower compared to the vehicle group, while the expression of nNOS was similar to sham operated levels (Fig.1).

Discussion: Some reports have demonstrated transient elevation of NO production and nNOS expression following spinal cord contusion injury. In contrast, the present study found a reduction in nNOS protein expression following SCTI and no changes in NO level. We hypothesize from these results that arginase induction may be involved in the injury response mechanism. Arginase could regulate NO production by competing with NOS for the same substrate, L-arginine. Bussiere et al. recently reported that arginase activation could also inhibit NOS translation (1). In the present study we report for the first time an increase in arginase activity and protein expression following SCTI. This raises the possibility that the observed lack of change in NO level might be due to the consumption of L-arginine substrate by arginase. Protein nitrotyrosine and subsequent cell death are usually caused by peroxynitrite following the reaction of superoxide with NO. In the present study, nitrotyrosine levels and TUNEL staining were significantly increased despite no change in the NO level. In agreement with these results, others have reported that NOS can simultaneously generate superoxide under conditions of decreased L-arginine (2) and that inhibition of nNOS can enhance superoxide production (3). Based on these findings, we suggest that nNOS levels decrease following the depletion of L-arginine by arginase, hence generating superoxide. In support of this, administration of arginase inhibitor was observed to attenuate the increases in nitrotyrosine and cell death and to recover the down-regulation in nNOS protein expression. In conclusion, the regulation of arginase may provide a novel approach of preventing cell death following SCTI by reducing the amount of oxidative-nitrosative stress.


Fig. 1 Western blot analysis of nNOS (A) and nitrotyrosine (B) expression

nNOS expression was significantly decreased in the vehicle group compared to the sham operated control group. In the treatment group, nNOS expression recovered to the level seen in the sham operated control group (A). Nitrotyrosine expression was significantly higher in the vehicle group compared to the sham operated control group. However, in the treatment group it was significantly lower compared to the vehicle group (B). * P < 0.05, ** P < 0.01