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Superoxide radicals play important roles in the pathogenesis of spinal cord injury

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To investigate a possible role of supreoxide dismutase (SOD) in the pathogenesis of spinal cord injury (SCI), the change in the circulatory status in an experimental SCI model of rats was examined. The blood pressure (BP) of the SCI group decreased markedly by compression or head-up-tilt (60°). The extent of the BP decrease was diminished by the administration of a long acting SOD (SM-SOD) prior to injury. Compression, tilting and the administration of SM-SOD increased the sensitivity of resistant arteries to acetylcholine. SM-SOD treated rats had a significantly lower mortality than was found in untreated rats. These findings indicate that superoxide radicals may contribute to the pathogenesis of spinal cord injury and orthostatic hypotension caused by SCI.

Keywords: spinal cord injury; hypotension; orthostatic hypotension; superoxide radicals; EDRF (endothelium-derived relaxing factor); experimental spinal cord injury

Introduction

Cardiocirculatory changes, especially orthostatic hypotension, have been known to occur frequently in patients with a spinal cord injury. These blood pressure aberrations are common and are a serious complication of clinical spinal cord injury.[1,2] It has been reported that the loss of function or adaptation of the autonomic nervous system and the body's homeostasis in patients with SCI lead to this postural hypotension, but the true cause is uncertain.[3,4] Although many investigators demonstrated that disorders of the spinal cord injury were associated with abnormalities of free radicals that affected membrane lipids in ischaemic tissues[5–7] since the concept was introduced by Demopoulos,[8] there were few reports that the influence of free radicals had been investigated from the standpoint of the hypotension.[9] In this study, we examined the effects of SOD on blood pressure and mortality in rats after spinal cord compression.

Materials and methods

Animals

Adult, pathogen-free, male Wistar rats (Nihon SLC, Hamamatu, Japan), weighing 270–350 g, were used in all experiments after fasting for 16 h. A femoral artery was cannulated and subsequently connected to a microtransducer for the measurement of blood pressure. Arterial resistance was determined by the intravenous administration of acetylcholine (Ach, 10^{-6} M, 0.2 ml).

Synthesis of SM-SOD

SM-SOD was synthesized as has been described previously by linking 2 mol SM (styrene co-maleic acid butyl ester, Curare) per mol SOD (Sigma Chemicals, St Louis, MO, USA).[10] 10 min before spinal cord injury and/or during experiment, several animals were administered SM-SOD (10 mg kg^{-1}) dissolved in 0.4 ml saline from the left femoral vein. Control animals received 0.4 ml saline solution.

The model of spinal cord injury and orthostatic hypotension

Under pentobarbital anaesthesia (45 mg kg^{-1}), the animals had a laminectomy with a surgical airtome at the level of T12 (thoracic spine T12). SCI was induced by placing a 20 g weight extradurally on to the spinal cord at 12 level for 15 or 60 min. The weight used had a round concave lower surface of 6 mm diameter, and a square top of 2 mm^2 area, which exactly fitted the spinal cord.[7] One day after the SCI, orthostatic hypotension was induced by a 60° head up tilt for 15 min.

Statistical analysis of experimental data

Data are expressed as mean ± SD. The P values and statistical significance are indicated as computed by an analysis of variarance (ANOVA). The non-parametric data of depressor action of Ach were analyzed by the Kruskal-Wallis test. A P value of less than 0.05 was considered significant.

Result

The typical pressor response of an untreated rat, subjected to compression injury for 60 min, is shown in Figure 1a. At the time of spinal cord injury, a hypertensive spike occurred. This transient hypertension was followed by profound hypotension. Improvement of the hypotension was observed in rats pretreated with SM-SOD (Figure 1b), while there was no significant difference between untreated- and SM-SOD-treated rats in hypertensive phase.

One day after spinal cord injury with a 20 g weight compression for 60 min, blood pressure of the untreated rats was unstable as compared with SM-SOD-treated animals (Figure 2). Figure 3 shows the effect of SM-SOD on the change in blood pressure during tilting. Control animals were subjected to laminectomy only. Hypotension in SM-SOD-treated rats occurred minimally as compared with the untreated group. The pressor response was similar to that found in the control group. As shown in Figure 4, compression and tilting increased the sensitivity of resistant arteries to Ach. Figure 5 shows the time required for the recovery of the blood pressure after Ach administration. There

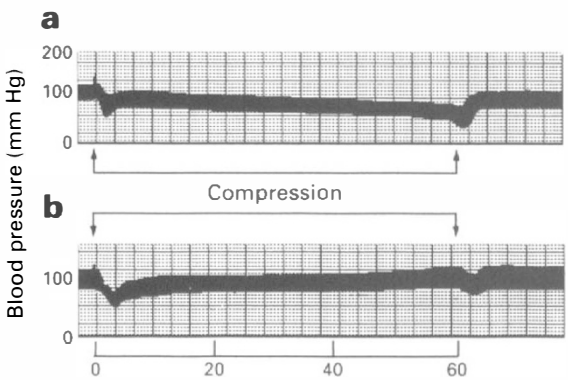

Figure 1 Effect of SM-SOD on the blood pressure of animals during and after compression. (a) untreated (injured) rat, (b) SM-SOD-treated rat 10 min before injury

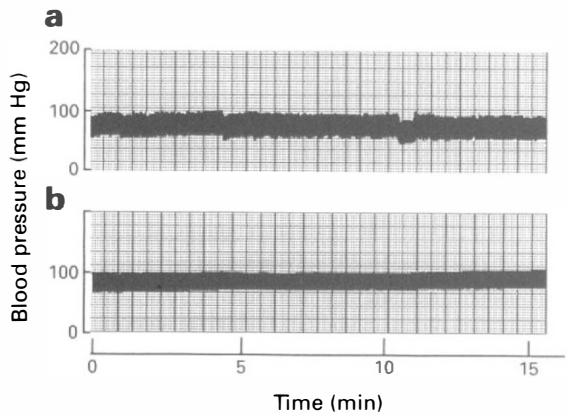

Figure 2 Blood pressure 1 day after spinal cord injury with a 20 g weight compression for 60 min. (a) untreated rat, (b) SM-SOD-treated rat

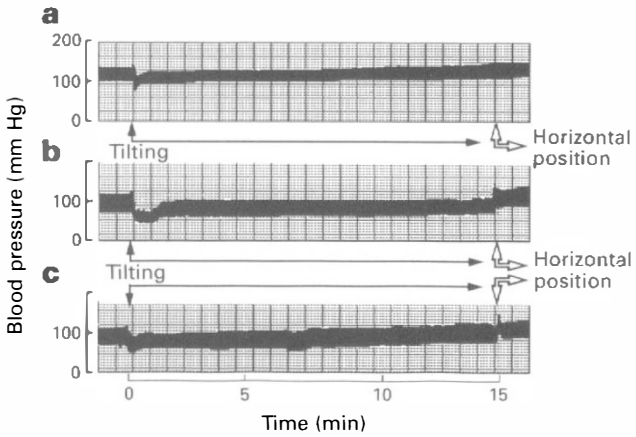

Figure 3 Effect of SM-SOD on the change in blood pressure during tilting 1 day after spinal cord injury. The black arrows indicate 60° head up tilt and the white arrows indicate repositioning horizontal level. (a) Control, control animals were subjected to laminectomy only, (b) untreated rat, (c) SM-SOD-treated rat

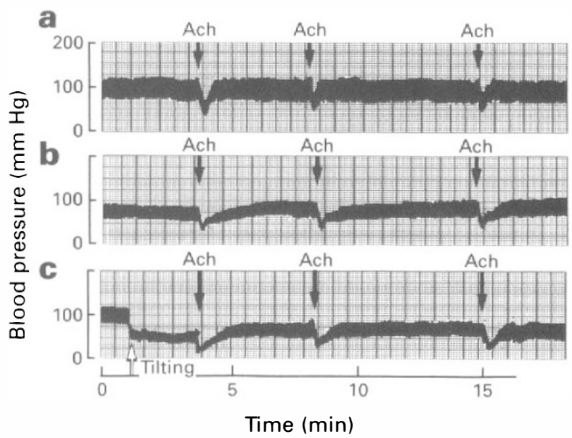

Figure 4 Depressor effect of acetylcholine. The black arrows indicate administration of ACh (10^{-6} M, 0.2 ml) and the white arrow indicates tilting. (a) Control, (b) untreated rat during compression, (c) untreated rats during tilting

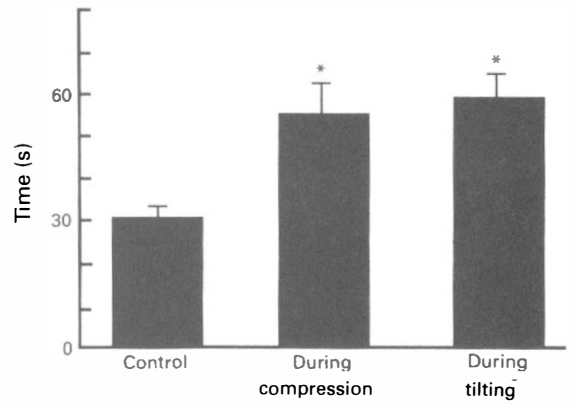

Figure 5 Depressor action of Ach. Data show the time required for the recovery of the blood pressure after Ach administration. The data are expressed as mean ± SD ($n = 15$). *$P < 0.01$ compared to control

was a significant difference between control and compression/tilting animals. The increased sensitivity was more marked in animals administered with SM-SOD during compression and tilting (Figure 6). The mortality within 48 h after spinal cord injury with a 20 g weight compression for 1 h was markedly decreased by treating animals with SM-SOD (Table 1).

Discussion

Spinal cord injury consistently evokes a transient 4- to 5-min rise in systemic blood pressure (hypertensive phase), followed by prolonged hypotension (hypotensive phase) as shown in Figure 1. This result is consistent with reports in earlier works.[11,12] Young *et al*[13] suggested that the hypertensive phase is composed of two components, an early one contributed by the thoracic sympathetic ganglia and a later one mediated by the adrenal glands in cats subjected to a contusion injury of the thoracic spinal cord. We found that SM-SOD treatment before compression injury ameliorated not the hypertension but the hypotension, as well as orthostatic hypotension and mortality. These findings at least show that SM-SOD does not act on the thoracic sympathetic ganglia and/or the adrenal glands. What are the possible mechanisms of the SM-SOD effects? The first is that SM-SOD may act on central opiate receptors as well as naloxone which is an opiate antagonist.[14] Holaday and Faden[15] demonstrated that naloxane improved the hypotension and hypoventilation which occur in the rat spinal shock model mediating by a blockade of endorphin effects at opiate receptors in the central nervous system. Naloxone also has been shown to be effective in improving neurological recovery following impact trauma to the spinal cord in cats.[16] In addition, naloxone significantly improved post-traumatic blood flow in both gray and white matter, and preserved somatosensory-evoked potentials following the trauma.[17]

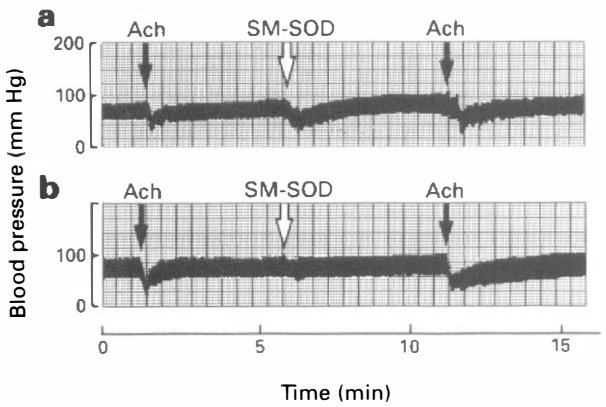

Figure 6 Effect of SM-SOD on the change in blood pressure. The black arrows indicate administration of ACh (10^{-6} M, 0.2 ml) and the white arrows indicate that of SM-MOD (10 mg kg^{-1}, 0.4 ml). (a) Untreated rat during compression, (b) untreated rat during tilting

Table 1 Effect of SM-SOD on the mortality of the injured animals within 48 h after SCI

Injured	*+SM-SOD*
4/8 (50%)	1/6 (16.7%)

Another possibility is that scavenging free radicals may lessen spinal cord injury. Demopolus *et al*[8] have supported their free radical hypothesis of spinal cord damage with studies of free radical or peroxidation chemistry in traumatised spinal cord tissue. Pietronigoro *et al*[18] and Saunders *et al*[19] reported that antioxidants, such as vitamin C, vitamin E and selenium, have protective effects on post-traumatic ischaemia in the spinal cord. Naloxone has also been shown to inhibit iron-catalysed peroxidation in liposome.[20] It has been reported that SOD reduced post-ischaemic paraplegia in animals with an aortic occlusion.[21] In addition, SM-SOD is a long acting SOD which increases the circulatory half-life of this enzyme from 6 min to 6 h.[10] These data permit speculation that these agents may be acting, at least in part, through a common mechanism mediating the effects of spinal cord injury, by quenching the peroxidative reactions associated with this injury.

These investigations represent the first assessment of orthostatic hypotension in spinal-injured rats. We measured orthostatic hypotension one day after SCI. Such acute measurements may not represent true long-term orthostatic hypotension in the rats. However, acute orthostatic hypotension may reflect spinal shock and other reflex changes in the injured spinal cord. Spinal shock lasts for minutes in frogs, 1–2 h in dogs and cats, days in monkeys, and several weeks in humans.[22] Thus, pressure changes in rats one day after SCI may represent not only acute but subacute consequences of the reflex compromise. Furthermore, the technique that we have described is useful for assessing both acute, subacute, and chronic orthostatic hypotension.

Orthostatic hypotension is a frequent complication in paralysed patients after SCI.[1,2] The symptoms associated with acute hypotension significantly interfere with the rehabilitation of patients with SCI. Furthermore, hypotensive manifestations may be present for several months, until patients develop tolerance to the low blood pressure.[3] Although it has been reported that the pathogenesis of orthostatic hypotension caused by SCI is complex and involves the cardiovascular, renal, neurological and the endocrine systems, the true cause is uncertain.[4,23]

Our data demonstrated that compression and tilting increased the sensitivity of resistant arteries to Ach and the increased sensitivity was more marked in animals administered with SM-SOD during compression and tilting. It has been known that Ach induces vasodilation by releasing endothelium-derived relaxing factor (EDFR), since the concept was introduced by Furchgott.[24] Gryglewski *et al*[25] demonstrated that EDRF is protected from breakdown by superoxide dismutase and superoxide radicals contribute significantly to the

instability of EDRF. Recently Nakazono *et al*[26] indicated that superoxide radicals in and around vascular endothelial cells play critical roles in the pathogenesis of hypertension in the spontaneously hypertensive rat. These findings are consistent with the hypothesis that EDRF and superoxide radicals may contribute to the pathogenesis of orthostatic hypotension caused by SCI. It is not known whether EDRF and/or superoxide radicals underlie the pathogenesis of this postural hypotension in humans. However, allopurinol, a xanthine oxidase inhibitor, decreases blood pressure in patients with essential hypertension and hypotension is one of the frequently observed side effects of allopurinol.[27,28] Thus, superoxide radicals and EDRF may underlie the pathogenesis of orthostatic hypotension in human subjects.

As is discussed above, SOD may have different pharmacological specificities in SCI; one is amelioration of hypotension, orthostatic hypotension and mortality by the administration of SOD prior to SCI, and another is, by the administration of SOD during compression or tilting after SCI, acceleration of the sensitivity of resistant arteries to Ach, leading to prolonged hypotension.

Further experiments are required to clarify the pathogenesis of SCI and the mechanism of orthostatic hypotension and to develop therapeutic agents for the treatment of this postural hypotension.

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