Short Communication

N-Acetylcysteine Fails to Protect Rats from Acrylamide Neurotoxicity

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Acrylamide monomer can impair the central and peripheral nervous system in humans and experimental animals

N-Acetylcysteine (NAC), a thiol-containing compound, has been used against acetaminophen overdose due to its potential to stimulate the synthesis of glutathione in the liver

In addition, NAC can directly reduce the level of reactive oxygen species such as hydrogen peroxide, hydroxy radical and hypochlorous acid, and also affect the gene regulation of transcriptional factors including AP-1 and NF-κB. On the other hand, it has been reported that NAC promotes survival of trophic factor-deprived PC12 cells and sympathetic neurons. NAC also enhances neuronal survival in the animal models of transient forebrain ischemia and of lower motor neuron degeneration. These findings suggest that NAC may exert neuroprotective actions. To our knowledge, however, no in vivo effects of NAC on the neurotoxicity of chemicals have been reported. We therefore examined whether or not NAC can suppress the neurotoxicity of acrylamide in rats.

Materials and Methods

Acrylamide (Wako Pure Chemicals, Osaka, Japan) and NAC (Sigma Chemical Co., St. Louis, MO, USA) were dissolved in 0.85% NaCl. Male Wistar rats weighing 170–190 g were divided into 4 groups each consisting of 8 animals. After 14 days for adaptation to the environment, the rats were administered chemicals from day 15 to 19 and 22 to 24 as follows; (1) saline, intraperitoneally plus saline, subcutaneously, (2) saline, intraperitoneally plus acrylamide 50 mg/kg/day, subcutaneously, (3) NAC 0.5 g/kg/day, intraperitoneally plus acrylamide 50 mg/kg/day, subcutaneously, and (4) NAC 1.0 g/kg/day, intraperitoneally plus acrylamide 50 mg/kg/day, subcutaneously. After 8 injections, all the rats were allowed to recover from day 25 to day 60. Animals had free access to chow and water throughout the experiment.

To evaluate the neurological deficit, the distance between the toe pads of bilateral hind limbs was determined when the rat landed from a height of 30 cm (landing foot spread, LFS) as described previously. LFS and body weight were determined every day, and the behavior of each rat was carefully observed.

In another set of experiments, rats were killed at 24 h after 8 injections, and the level of glutathione in the brain was determined by the method of Sedlak and Lindsay. The protein concentration was determined by DC Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analyses of LFS and body weight in 4 groups were done by the Kruskal-Wallis test followed by Dunn’s test and by one-way analysis of variance (ANOVA) followed by Bonferroni’s test, respectively. Comparisons of LFS at two time points in a group were examined by the Mann-Whitney test. Differences at p<0.05 were considered statistically significant.

Results

Rats injected with acrylamide developed ataxia and weakness of the hind limbs on day 22 or 23 (after 6 or 7 injections). When compared to day 15 (the first day of injection), the LFS of rats given acrylamide had increased significantly by day 22 (7.3 ± 0.6 cm, mean ± S.D., n=8, p<0.001), peaked on day 29, and returned to the basal level by day 45 (Fig. 1). On day 29 when the neurological impairment evaluated with LFS was most marked, there was no significant difference in the LFS between rats given acrylamide alone and those given acrylamide plus 0.5 or 1.0 g NAC/kg/day (Fig. 1). On days 45 and 60, no statistically significant differences in LFS were found among 4 experimental groups (p>0.05 by ANOVA).

Significant decreases in body weight were observed between controls and rats given acrylamide with or without NAC administration on day 29 (Fig. 2). Thereafter, the body weight of rats given acrylamide only did not differ from that of the controls, but the rats given 1.0 g NAC/kg/day still showed a slight decrease in body weight.

The level of glutathione in the brain 24 h after 8 injections was 14.2 ± 1.4, 14.7 ± 1.8, 14.2 ± 2.4 and 15.1 ± 1.6 nmol/mg protein (mean ± S.D., n=8) in the control, rats given acrylamide alone, rats given acrylamide plus 0.5 g NAC/kg/day, and those given acrylamide plus 1.0 g NAC/kg/day, respectively. There was no significant difference in the brain glutathione level among these 4 groups (p>0.05 by ANOVA).

Discussion

Although the dose of NAC used in the present study (0.5 or 1.0 g/kg) was much higher than 326 mg/kg, a dose of NAC that has been demonstrated to improve neural survival after transient forebrain ischemia and to reduce the lethal dose of acrylonitrile in rats, the neurological impairment of rats given acrylamide was not ameliorated. Furthermore, the decrease in body weight, a general toxic sign of acrylamide, was found in rats given a higher dose of NAC (1.0 g/kg). Consistent with these observations, the inhibition of creatine kinase activity in the brain, which is a possible neurotoxic indicator of acrylamide intoxication, was also seen in mice.
given NAC (data not shown). Because NAC has been reported to partially improve neuronal survival in the CA1 region of the hippocampus after forebrain ischemia\(^7\), the possibility cannot be excluded that the neuroprotective effects of NAC might be limited to a certain cerebral region or regions.

The levels of glutathione in the brain of rats given acrylamide with or without NAC administration were not different from that in the control. These findings suggest that the brain glutathione level does not play a major role in the development of acrylamide-induced neurological impairment (increase in LFS). It has been reported that treatment with NAC (200 mg/kg/day, 10 days) did not increase the lung glutathione level and lead to more respiratory distress in rats exposed to 85% O\(_2\) for 7 days\(^1\). It is not known why the repeated administration of NAC to rats did not increase the glutathione level in tissues.

We have previously found that NAC strongly suppresses the cytotoxicity of cadmium, one of the sulfhydryl-reactive heavy metals, in LLC-PK\(_1\) cells\(^14\). This NAC-induced protection against cadmium cytotoxicity is not due to a change in the glutathione level but mainly due to lowered uptake of cadmium into the cells, indicating interaction of NAC with cadmium\(^14\). However, the present study suggests that NAC may not interact with acrylamide.

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References


