Letter to the Editor

Acrylamide Alters Cytoskeletal Protein Level in Rat Serum*

YU Su Fang[1], SONG Fu Ying[1], YI Chao[2], YANG Xi Wei[1], LI Guo Zhen[1], ZHANG Cui Li[1], ZHAO Xiu Lan[1], and XIE Ke Qin[1,#]

To study biomarker of acrylamide (ACR) induced neuropathy, Wistar rats received 20 or 40 mg/kg of ACR by *ip* injection and the levels of light neurofilament (NF-L), middle NF (NF-M), heavy NF (NF-H), β-actin, α-tubulin, and β-tubulin proteins in serum were evaluated using both SDS-PAGE and Western blotting. Compared to controls, NF-L and NF-M decreased, however α-tubulin, β-tubulin, and β-actin increased (*P*<0.01). This result suggested that the change of these cytoskeletal proteins might be relevant to the neurotoxicity of ACR. Some of the cytoskeletal proteins in serum might be used as marker of biological effect in ACR induced neuropathy.

Acrylamide (ACR) is used universally in industrial process. It is also present in certain foods prepared at very high temperature[1]. Overexposure of humans and laboratory animals to monomer ACR has produced neurotoxic syndromes such as ataxia, skeletal muscle weakness, and weight loss[2]. Early morphological studies revealed that ACR induced neuropathy was associated with nerve damage characterized by multifocal paranodal swellings of preterminal distal myelinated axons and consequent degeneration[2-3]. However, the molecular mechanisms of ACR-induced neurodegenerative disorders have not been completely elucidated. Our previously study showed that cytoskeletal protein was significantly changed in both nerves and plasma of ACR-induced neuropathy[4-6].

Cytoskeletal protein is an abundant protein component of neuron and is relevant to the movement, reproduction, and death of the cell, which is considered as the hallmark of a specific neuron degenerative process. This study aims to detect cytoskeletal proteins in serum of ACR-induced animal model and to initially explore the changes of these proteins that might be relevant to the peripheral neurotoxicity of ACR. It might provide evidence supporting the use of cytoskeletal proteins for the detection of peripheral neurotoxicity of ACR.

Male Wistar rats were randomly assigned to 3 groups (*n*=9 rats per group). To evaluate the changes in cytoskeletal proteins, two groups were exposed to ACR at different dosages, i.e. 20 or 40 mg/kg·bw by *ip* injection (3 times a w, during 8 w) to produce sub-chronic induction of neurological deficits. ACR was dissolved in 0.9% saline and administered at 1 mL/kg·bw. Control group rats received an equivalent volume of 0.9% saline by *ip* injection. Gait scores were assessed according to LoPachin method[7].

After 8 weeks of ACR administration, all the rats at the 40 mg/kg·bw group were severely affected, while the rats at the 20 mg/kg·bw dose-rate group were only slightly affected. Both groups of ACR-treated rats showed abnormal behavior, such as lethargy, reduction in locomotor activity, and some ataxia. With the development of the experiment, the abduction of hind limbs, foot splay, and hopping gait were observed in the low dosing group, while dragging of the feet along the floor and hind limb paralysis were observed in the high dosing group (Figure 1).

Gait scores from an initial 1±0 (a normal gait) increased to 4±0 (a severely abnormal gait) at a high dose of ACR and relatively slowly increased to just 2±0 (a slightly abnormal gait) at a low dose of ACR (Figure 2A). ACR postponed body weight gain in a dose-dependent manner, i.e. only the high ACR dosage caused a significant retardation of normal weekly weight gain (Figure 2B). The gait scores increased to the highest 4 points and body weight reduced remarkably in the high dose ACR group at the end of the experiment. According to the criterion[7], it is shown that there is a severe nerve injury in the high dose group.

Wistar rats were sacrificed by decapitation after 8 w of ACR administration. About half of the blood initially collected was discarded and the remaining half was rapidly collected into plastic tubes. The blood was centrifuged at 3 000 ×g for 20 min to obtain the serum. The serum was separated carefully and mixed with serum buffer containing 10 mmol/L Hepes, pH 6.8, 50 mmol/L NaF, 1 mmol/L EGTA, 1 mmol/L, 2 mmol/L levamisol, and 1 mmol/L EDTA phenylmethylsulfonyl fluoride (PMSF), and then stored at -80 °C. The method of measurement for cytoskeletal proteins using both SDS-PAGE and Western Blotting has been previously described by YU[5]. The sampling protein amounts per lane of NF-L,

doi: 10.3967/ bes2013.023

1. School of Public Health, Shandong University, Jinan 250012, Shandong, China; 2. Guangming New District CDC of Shenzhen, Shenzhen 518106, Guangdong, China

NF-M, NF-H, α-tubulin, β-tubulin, and β-actin were 10, 20, 25, 25, 25, 20 μg, respectively.

The three major constituents of the neuronal cytoskeleton are NF, actin, and tubulin. These proteins are responsible for creating the intracellular scaffolding of the cell. As shown in Western Blotting and quantitative analysis, it was demonstrated that the levels of some cytoskeletal proteins had changed in ACR-induced rats serum (Figure 3). Generally, the NFs decreased (Figure 3A), whereas α-tubulin, β-tubulin, and β-actin increased (Figure 3B).

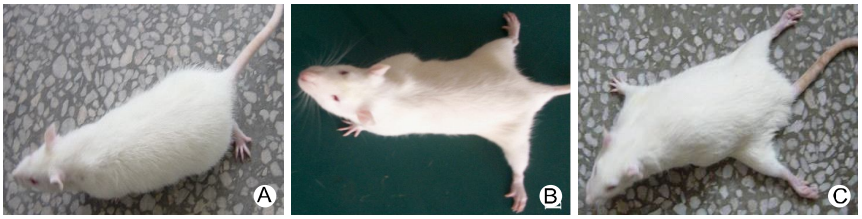

Figure 1. Weakness of limbs during acrylamide intoxication. Compared with control rats (A), rats in low dose-rate exposure group developed an unsteady walking pattern with abduction and external rotation of hind limbs (B, 8 w of low dose ACR treatment), rats in high dose-rate exposure group developed hind limb paralysis and dragging of the feet along the floor (C, 8 w of high dose ACR treatment).

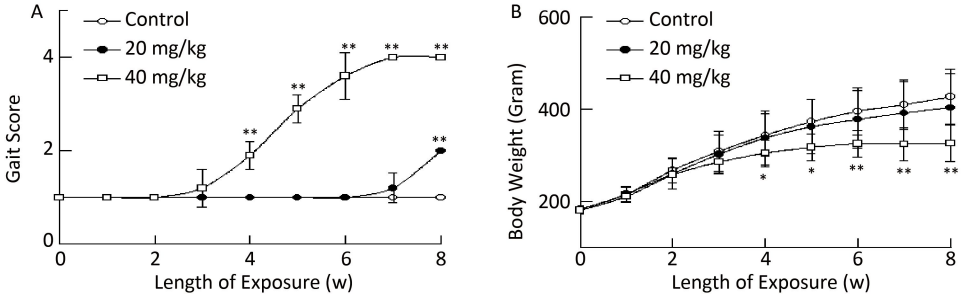

Figure 2. Effects of ACR on mean±SD gait scores (A) and body weight in grams (B). The asterisk indicated statistical difference between ACR exposure groups and the control group ($^{*}P<0.05$, $^{**}P<0.01$). The mean body weight (325.7±40.1) of higher dose group was significantly lower than that of both the control group (426.5±59.3) and lower dose group (403.4±73.6) on the 8th week exposure period (B).

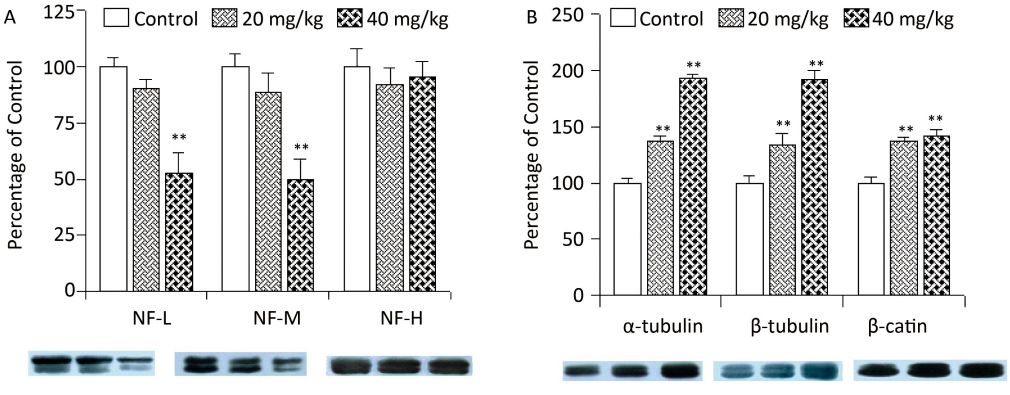

Figure 3. Effects of ACR on levels of cytoskeletal proteins in serum. The results were presented as a mean percentage of the corresponding control±SD (*n*=9), and representative immunoblots were also shown in the figure. The asterisk indicated statistical difference between ACR exposure groups and the control group ($^{**}P<0.01$). Compared with the control, both NF-L and NF-M subunits levels decreased significantly in high ACR group (A); the levels of α-tubulin, β-tubulin, and β-actin increased significantly in serum in both low and high ACR groups (B).

Compared with the control, the level of three subunit NFs reduced in both high and low ACR groups, but only NF-L and NF-M lessened significantly in the high ACR group (P<0.01, Figure 3A). NF is assembled from three polypeptide subunits and abundant in mature axons. Axons were synthesized in the cell body and assembled as heteropolymers requiring NF-L with either NF-M or NF-H[8]. The essential role of NF is to establish and maintain the diameter of mature axon that is the principal determinant of conduction velocity of impulses along the axon. NF dysfunction has been linked to the etiology of various neurodegenerative disorders, and berrant NF accumulation has been also found in Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis[9].

Two of these cytoskeletal proteins, tubulin and actin, also act as tracks that bind and activate transport mechanisms that guide intracellular organelles to their destinations. Recent studies suggested that both actin and MTs were key regulators of many cell morphological events and most likely played important roles in neurite initiation. Actin and MTs can interact with both cytoskeletal networks, such as microtubule associated proteins, and are strong candidates to mediate this crosstalk. As shown by Figure 3B, the level of α-tubulin, β-tubulin, and β-actin proteins was increased by ACR in both high and low dose groups (P<0.01). Actin is the major component of the microfilaments, which are the major components of the presynaptic terminals and dendritic spines, probably to form and organize these cytoplasmic specializations. Microtubules (MTs) are dynamic, polarized structures that are formed by the polymerization of heterodimeric complexes of α- and β-tubulin. Its polarity is relevant to its transport processes and kinesins, direct transport processes to the plus-end, and dyneins to the minus-end.

This result showed that ACR can affect cytoskeletal proteins. As for the direction of changes, it was seen that ACR decreased NF-L, NF-M, NF-H but increased α-tubulin, β-tubulin, and β-actin. As for the range of changes, ACR increased α-tubulin and β-tubulin the most, by more than 90%, while it affected NF-H only a little without statistical significance.

Informative biomarkers detectable in serum or plasma are convenient for researchers and clinicians since blood is routinely and much more easily obtained than cerebrospinal fluid (CSF) or other tissues. Our results showed that the levels of some cytoskeletal proteins in serum changed in ACR-induced groups. As mentioned above, these proteins were related with the axonal reaction and neuronal function. Especially, since NF was found only in neurons, its changes suggested that neurons and/or axons might be damaged or destroyed. In some nervous diseases with axonal degeneration, cytoskeletal proteins were detected in CSF. Several studies have indeed shown that the concentration of NF-L is increased in CSF of patients with parkinsonian syndromes and multiple sclerosis (MS)[10]. Actin and tubulin concentrations also increased in CSF of patients with progressive MS.

We observed that most of the detected proteins' levels, especially NF subunits, decreased at a high ACR dose and the changes of α-tubulin and β-tubulin were different from other proteins. From the results of body weight and gait scores, we presumed that ACR administrated in this study induced the nervous injury at a high dose. Meanwhile, the level of some cytoskeletal proteins in serum has changed obviously, so that the changes of these proteins in serum might be relevant to the neurotoxicity of ACR. These results are generally in accordance with the changes in the sciatic nerve and plasma[5-6]. However, our precious study showed that ACR increased NF-L and NF-M in the supernatant, whereas it decreased NF-L and NF-M in the pellet of cerebrum, which is not consistent with the levels in serum. This study is only a preliminary exploration of the effect of ACR on the changes of cytoskeletal protein in serum. More research needs to be done to determine its underlying mechanism.

The results suggested that the changes of these cytoskeletal proteins might be relevant to the neurotoxicity of ACR. Some of the cytoskeletal proteins in serum might be used as the biological effect markers of ACR induced peripheral neuropathy.

*This work was supported by the National Natural Science Fund of China (No. 30872088) and Technology Development Plan of Shandong Province (No. 2012GSF11854).

#Correspondence should be addressed to XIE Ke Qin (PhD, professor). Tel: 86-531-88382132, Fax: 86-531-88382553, E-mail: keqinx@sdu.edu.cn

Biographical note of the first author: YU Su Fang, female, born in 1961, Doctor, professor, majoring in industrial toxicology.

Received: May 2, 2013;
Accepted: June 20, 2013

REFERENCES

1. Sathya N Prasad and Muralidhara. Evidence of acrylamide induced oxidative stress and neurotoxicity in Drosophila melanogaster-Its amelioration with spice active enrichment: Relevance to neuropathy. Neuro Toxicology, 2012; 33, 1254-64.
2. Deng H, He S, and Zhang S. Quantitative measurements of vibration threshold in health adults and acrylamide workers. Int Arch Occup Environ Health, 1993; 65, 53-6.
3. Zhang L, Gavin T, David S, et al. Role of the Nrf2-ARE pathway

in acrylamide neurotoxicity. Toxicology Letters, 2011; 205, 1-7.
4. Yu SF, Zhao XL, Zhang TL, et al. Acrylamide-induced changes in the neurofilament protein of rat cerebrum fractions. Neurochem Res, 2005; 30, 1079-85.
5. Yu SF, Song FY, Yu JX, et al. Acrylamide alters cytoskeletal protein level in rat sciatic nerves. Neurochem Res, 2006; 31, 1197-204.
6. Yi C, Xie KQ, Song FY, et al. The changes of cytoskeletal proteins in plasma of acrylamide-induced rats. Neurochem Res, 2006; 31, 751-7.
7. LoPachin RM, Ross JF, Reid ML, et al. Neurological evaluation of toxic axonopathies in rats: Acrylamide and 2,5-hexanedione. Neuro Toxicology, 2002; 23, 95-110.
8. Lariviere RC, Julien JP. Functions of intermediate filaments in neuronal development and disease. J Neurobiol, 2004; 58, 131-48.
9. Cassie SM and Robert HL. Cargo distributions differentiate pathological axonal transport impairments. Journal of Theoretical Biology, 2012; 300, 277-91.
10. Sara B, Lena E, Lisette S, et al. Amyloid-related biomarkers and axonal damage proteins in parkinsonian syndromes. Parkinsonism & Related Disorders, 2012; 18, 69-72.