Tr. J. of Medical Sciences
28 (1998) 47-51

Piraye YARGIÇOĞLU[1]
Aysel AĞAR[2]
Ümit Kemal ŞENTÜRK[2]
Nimet İzgüt UYSAL[2]
Derya KILIÇ[1]

The Effect of Pre-and Postnatal Cd Exposure on Conduction Velocity in Sciatic Nerve

Received: January 29, 1996

Department of [1]Biophysics, [2]Physiology
Faculty of Medicine, Akdeniz Üniversity,
Antalya-Turkey

Abstract: The purpose of the study has been to investigate the effect of pre-and postnatal Cd exposure on conduction velocity of sciatic nerve.

Pregnant Swiss albino rats were divided into three groups as control (C), cadmium (Cd) and non-cadmium (NCd) groups. Control animals received tap water while rats in Cd group received Cd as $CdCl_2$ in their drinking water during the experimental period. The mothers of the NCd group were given Cd during their pregnancy but given tap water after birth. Twenty-two days after birth (postnatal day 22) the rats were separated from the mothers. The present study was performed on 80 rats which were divided into C, NCd, Cd_1 and Cd_2 groups, each including 20 rats. Cd_1 group received $CdCl_2$ water for an additional 8 days. On postnatal day (PND) 30, conduction velocities and amplitudes of compound action potentials were determined from sciatic nerves of this group. The other rats were continued to be treated with Cd (Cd_2 group) or tap water (C and NCd groups) for an additional 38 days. On PND, 60, the same measurements wer made for these groups as mentioned above.

The means of the peripheral conduction velocities decreased significanty in all the Cd-treated groups compared with control group. The mean of the compound action potential amplitudes was significantly decreased only in Cd_2 group compared with control group.

Introduction

Cadmium (Cd), one widely studied environmental contaminant, has been shown to cause functional disturbances in both the peripheral nervous system (PNS) and central nervous system (CNS) due to its toxic effects on various tissues (1-4). Among the various hazardous effects of Cd are inhibition of bioamine uptake, Na^+-K^+ ATP ase and voltage-dependent Ca^{++} channels which lead to alterations in the functions of transmitter systems (5-8). On the other hand, the major effect of Cd exposure has been reported to increase lipid peroxidation by reducing antioxidative enzymes associated with the protective mechanisms against free radicals (9-13). It is well known that lipid peroxidation plays an important role in the mechanism of many pathologic disorders. Therefore, peroxidative effect of Cd has attracted considerable interest in the last decade.

The neurotoxic effects of Cd depend on developmental stage (3,14). During pregnancy, the placenta partially protects the fetus from direct effect of Cd by accumulating it (4,15). But Cd enters the central nervous system of developing animal with relative ease because of immature blood brain barrier (3, 14, 16, 17). So brains of growing rats are more sensitive to the toxic effect of Cd than the brains of adults.

Therefore, the present study was undertaken, firstly, to investigate the effect of pre-and postnatal Cd exposure on nerve conduction velocity. A second goal was to evaluate the role of free radicals in the mechanism of Cd toxicity by examining the relationship between lipid peroxidation and nerve conduction velocity changes.

Material and Methods

Preparation of animals: Adult healthy Swiss albino rats were mated (two females with each male). Pregnancy was tested by vaginal smear test. Pregnant rats were removed and kept in separate cage. They were divided into three goups: Control (C), cadmium (Cd) and non-cadmium (NCd) groups. Control animals were fed with normal food and tap water ad libitum, the rats in the Cd and NCd groups received 15 ppm of cadmium as $CdCl_2$ in their drinking water during the pregnancy. Following partirution, the Cd group re-

Table 1. The table shows how many offspring were taken from each dams within each group.

Dams/litters	Control			NCd			Cd_1 (One Month)			Cd_2 (Two Months)		
	A	B	C	A	B	C	A	B	C	A	B	C
1	8	8	3	3	3	3	2	2	2	1	1	1
2	9	9	3	2	2	2	3	3	3	2	2	2
3	9	8	2	3	3	3	3	3	3	3	3	3
4	8	8	3	3	3	3	3	3	3	3	3	3
5	7	7	2	1	1	1	2	2	2	3	3	3
6	7	6	2	2	2	2	3	3	3	3	3	3
7	8	8	3	3	3	3	1	1	1	3	3	3
8	8	8	2	3	3	3	3	3	3	2	2	2

A: The number of offspring

B: The number of living animal

C: The number of offspring were taken

ceived $CdCl_2$-water while the NCd and C groups were given tap water. The age of rats was recorded as zero day on the day of birth. At the end of 22 days (postnatal day 22), the rats were separated from the mothers. The present study was performed on 80 rats which were divided as control (20), NCd (20), Cd_1 (20), Cd_2(20). Each group including 20 rats which were taken from eight mothers. Because of a high incidence of pups of dams exposed to Cd, there were only one, two or three pups with each mother (Table.1). Cd_1 group received $CdCl_2$-water for an additional 8 days. On postnatal day (PND) 30, rats of Cd_1 group were deprived of food 24th and they were anaesthesied with diethylether (Merck) and all recordings were made under anaesthesia.

The other rats were continued to be treated with Cd (Cd_2 group) or tap water (C and NCd groups) for an additional 38 days. On PND 60, they were prepared for experiments as mentioned above.

Daily food and water consumption of every cage and weekly weight of each rats were recorded during the feeding period. The mean daily food and water consumption were estimated from the recorved values.

Recording: Stimulating and recording electrodes were placed on gastrocnemius and sciatic nerves, respectively. Bipolar compound action potentials (CAPs) were recorded from these extracelluler bipolar electrodes and amplified by a differential amplifier. Our electrodes and differential amplifier were designed in our department. The latencies, and amplitudes were measured. Conduction velocities were calculated by using nerve lengths and latency values of each rat.

Chemical Analysis: Blood samples were taken by cardiac puncture. The kidney and sciatic nerve tissues were then removed from rats. These tissues were used for cadmium analysis and sciatic nerve homogenates were used for thiobarbituric acid reactive substances (TBARS). Determination of TBARS mostly malondialdehyde (MDA), in body fluids and tissues is considered as an indicator of lipid peroxidation. TBARS were measured according to the procedure of Stocks et al. (18, 19). Protein contents were determined by the method of Lowry et al. (20) using bovine serum albumin as standard.

Cadmium Determination: Acid washed glassware was used during metal analysis. Tissues in glass tubes kept overnight at 65 centigrade, then about 100 mg of dry samples were digested in 1 ml of concentrated HNO_3 at 65 centigrade until they become clear. After appropriate dilution with deionized water, graphite furnace of atomic absorbtion spectrophotometry (Hitachi Z 8000) was used for cadmium analysis in blood and the clear digested samples (kidney and sciatic nerve). The procedure used for Cd anaysis was given in detail in a previous study (21).

Statistical Analysis: Differences of parameters between groups were tested by an analysis of variance (one-way $ANOVA_S$). Values of $p<0.05$ were considered significant.

Table 2. The means and standard deviations of cadmium, TBARS, sciatic nerve conduction velocity and compound action potential amplitude values.

	CADMIUM			TBARS	Peripheral Conduction Velocity	Amplitude
	Blood (µg/L)	Kidney (µg/g tissue dry weight)	Sciatic nerve (n mol/g tissue dry weight)	Sciatic nerve (n mol/g protein)	(m/s)	(V)
Control	3.42±1.28	0.066±0.014	0.111±0.039	233.99±65.17	69.23±8.58	0.24±0.09
NCd	7.00±2.18	0.43±0.42	0.334±0.058	281.51±48.14	49.16±6.47	0.21±0.06
	*F=21.09	*F=17.65	*F=102.55	*F=5.04	*F=60.17	*n.s.
	P<0.0002	P<0.0002	P<0.0001	P<0.02	P<0.0001	
Cd_1	6.68±1.88	1.41±0.65	0.412±0.13	310.12±62.8	48.87±5.49	0.21±0.07
	*F=25.7	*F=28.6	*F=120.14	*F=6.01	*F=79.82	*n.s.
(One Month)	P<0.0001	P<0.0001	P<0.0001	P<0.01	P<0.0001	**ns
	**n.s.	**F=12.1	**F=7.1	**n.s.	**n.s.	
		P<0.001	P<0.02			
Cd_2	11.2±2.56	3.43±1.34	0.613±0.17	420.13±83.57	40.88±7.67	0.14±0.03
(Two Months)	*F=76.92	*F=151.62	*F=75.23	*F=34.25	*F=97.0	*F=17.67
	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.001
	***F=14.28	***F=36.9	***F=6.5	***F=10.5	***F=5.6	***F=12.06
	P<0.001	P<0.001	P<0.01	P<0.01	P<0.03	P<0.002
	****F=20.26	****F=106.56	****F=22.08	****F=20.66	****F=5.55	****F=16.25
	P<0.0002	P<0.0001	P<0.0002	P<0.0003	P<0.03	P<0.001

* Experimental groups versus control

*** Cd_2 versus Cd_1

**Cd_1 versus NCd

**** Cd_2 versus NCd

Results

The mean Cd values of kidney, sciatic nere and blood samples of rats are summarized in Table 2. The mean Cd levels of kidney, sciatic nerve and blood were significantly increased in experimental groups compared with control group.

Lipid peroxidation was measured as the amount of TBARS. The data in Table 2 show that Cd treatment has caused an increase in lipid peroxidation in the sciatic nerves of Cd-treated rats.

The means and standard deviations of the peripheral conduction velocities and compound action potential (CAP) amplitudes are shown in the same table. The means of peripheral conduction velocities were decreased significantly in all the Cd-treated groups compared with control group.

The means of CAP amplitudes were not altered in NCd and Cd_1 groups, but significantly decreased in Cd_2 group compared with control group.

Discussion

Oral Cd exposure caused no significant changes in

both food or water intake of the rats at the any stage of experimental period. Therefore, the mean weight gain was not different in control and Cd exposed rats. However, as reported previously (4, 22), high incidence of fetal mortality occurred due to direct toxicity to the placenta (Table 1).

Our data clearly showed that Cd treatment caused a significant Cd accumulation in bloods, kidneys and sciatic nerves of Cd-treated groups compared with control group.But the concentration of Cd was increased in kidney much more than in blood and sciatic nerve. Because kidneys are major target organs to Cd exposure (4, 17). On the other hand, concentrations of Cd in blood and tissues of Cd-treated animals were increased dependent on the duration of Cd administration. So the mean conduction velocities of CAPs decreased in Cd-treated rats in accordance with the administration of Cd. Our finding is also consistent with previously reported data (23, 24).

The mechanims of Cd toxicity that contribute to decrease in nerve conduction velocity and amplitude of CAPs have been attributed to many factors. One of them have been related to channels. Experimental studies have revealed that there are three channels, which contribute to CAPs in the peripheral nerves. Cd is known to show unspesific blocking effects on calcium and sodium tetradotoxin (TTX)-resistant channels (23, 25-28). TTX – resistant action potentials were shown to originate from unmyelinated C fibers (25, 26). In the light of previous studies, the decrease in conductidon velocity and amplitude of CAPs due to increased levels of Cd in the sciatic nerve were resulted from the blocking of TTX-resistant, cadmium-sensitive sodium an calcium channels of C fibers.

Thes focus of the present study is to evaluate the peroxidative effect of Cd on electrophysiological properties of sciatic nerve. Therefore, TBARS were determined as an indicator of lipid peroxidation. Hence, TBARS level of sciatic nerves increased in Cd-treated animals in relation with Cd accumulation. The comparison of data among Cd-treated groups revealed that peripheral conduction velocities progressively decreased as TBARS contents of sciatic nerve increased. This result also demonstrated that there was a close relationship between conduction velocity and TBARS levels which was associated with the duration of Cd exposure.

Increased TBARS levels as observed in sciatic nerves of pre-and postnatal Cd administered rats are an appropriate indicator of oxidative stress. In the present study, the presence of significant increase of both Cd and TBARS contents in the sciatic nerves of Cd-treated rats strongly supports that enhanced lipid peroxidation caused the decrease in conduction velocities. Among hazardous effect of Cd, lipid peroxidation resulted from the failure of protective mechanisms due to Cd exposure plays an important role in the mechanism of Cd toxicity (9, 10, 12, 13, 29, 30). This is because peroxidative damage of membrane lipids leads to altered membrane structure and changes in physiological functions (9,29,30). In addition, damage of myelin specific lipids produced by oxidative stress caused hypomyelination (3, 14) and in turn slowing of the nerve conduction velocity.

In conclusion, according to our study, it can be concluded that there is a close relationship between slowing of nerve conduction velocity and Cd-induced lipid peroxidation.

References

1. Marlowe M, Errera J, Jacobs J. Increased lead and children burdens among mentally retarded children with borderline intelligence. Am. J. Men. Defic. 87: 477-83, 1983.
2. Thatcher RW, McAlester R, Lester ML. Evoked potentials related to hair cadmium and lead in children. Ann. N.Y. Acad. Sci. 425: 384-90, 1984.
3. Gulati S, Gill KD, Nath R. Effect of cadmium on lipid metabolism of brain: in vivo incorporation of labelled acetate into lipids. Pharmacol. Toxicol. 60: 117-9, 1987.
4. Tacey E, White K, Baggs RB, Miller RK. Central nervous system lesions in the wistar rat fetus following direct fetal injections of cadmium. Teratology, 42: 7-13, 1990.
5. Nechay BR, Saunders JP. Inhibitor characteristic of cadmium, lead and mercury in human sodium and potassium dependent adenosine triphosphatase preparations. J. Environ. Pathol. Toxicol. 2: 283-90. 1978.
6. Dhavale DM, Masurehar VB, Gridhar BA. Cadmium induced inhibition of Na-K ATPase activity in tissues of Crab Scylla Serrata (Forskal). Bull. Environ. Contam. Toxicol. 40 (5): 759-63. 1988.
7. Wiegand H, Uhlig S, Gotzsch U, Lohmann H. The action of cobalt, cadmium and thallium on presynaptic currents in mouse motor nerve endings. Neurotoxicol. Teratol. 42: 313-8, 1990.
8. Das KP, Das PC, Dasgupta S, Dey CD. Serotonergic-cholinergic neurotransmitters function in brain durin cadmium exposure in protein restricted rat. Biol. Trace Elem. Res. 36: 119-27, 1993.
9. Hussain T, Shukla GS, Chandra SU. Effects of cadmium on superoxide dismutase and lipid peroxidation in liver and kidney of growing rats: in vivo and in vitro studies. Pharmacol. Toxicol. 60: 355-8. 1987.

10. Shukla GS, Hussain T, Chandra SV. Possible role of regional superoxide dismutase activity and lipid peroxide levels in cadmium neurotoxicity: in vivo and in vitro studies in growing rats. Life Sci. 41: 2215-21, 1987.

11. Shukla GS, Srivastava RS, Chandra SV. Glutathione status and cadmium neurotoxicity studies in discrete brain regions of growing rats. Fund. Appl. Toxicol. 11: 229-35, 1988.

12. Ali MM, Shukla GS, Srivastava RS, Mathur N, Chandra SV. Effects of vitamin E on cadmium-induced locomotor dysfunctions in rats. Vet. Hum. Toxicol., 35(2): 109-11, 1993.

13. Sidhu M, Sharma M, Bhatia M, Awasthi YC, Nath R. Effect of cronic cadmium exposure on glutathione S-transferase and glutathione peroxidase activities in Rhesus monkey: The role of selenium. Toxicology, 83: 203-13, 1993.

14. Gulati S, Gill KD, Nath R. Effect of cadmium on lipid composition of the weanling rat brain. Acta Pharmacol. Et. Toxicol. 59: 89-93, 1986.

15. Bhattacharyya MM. Bioavailability of orally administered cadmium and lead to the mother fetus and neonate during pregnancy and lactation. An overview. Sci. Tot. Environ. 28: 327-42, 1983.

16. Gabbiani GD, Baic D, Deiziel C. Toxicity of cadmium for the central nervous system. Exp. Neurol., 18: 154-60, 1967.

17. Newland MC, Wendy W, Raymond NG, Baggs RB, Gentry GD, Weiss B, Miller RK. Operant behaviour in transition reflects naonatal exposure to cadmium. Teratology, 34: 231-41, 1986.

18. Stocks J, Dormandy TL. Autooxidation of human red cell lipids induced by hydrogen peroxide. Br. J. Hematol., 20: 95-111, 1971.

19. Stocks J, Offerman EL, Modell CB, Dormandy TL. The susceptibility to autooxidation of human red cell lipids in health and disease. Br. J. Hematol., 23: 713-24, 1972.

20. Lowry OH, Rosenbrough NJ, Far AL, Randell RJ. Protein measurement with Folin-phenol reagent. J. Biol. Chem., 193: 265-75, 1951.

21. Baselt RC. Analytical procedures for therapeutic drug monitoring and emergency toxicol. Biomedical Publications, Davis, Claifornia, 60-3, 1980.

22. Levin AA, Miller RK. Fetal toxicity of cadmium in the rat: Decreased uteroplacental blood flow. Toxicol. Appl. Pharmacol., 58: 297-306, 1981.

23. Bowers CW. A cadmium-sensitive, tetratoxin-resistant sodium channel in bullfrog autonomic axons. Brain Res., 340: 143-7, 1985.

24. Luscher C, Streit J, Lipp P, Luscher HR. Action potential propagation through embryonic dorsal root ganglion cells in culture II. Decrease of conduction reliability during repetitive stimulation. J. Neurophysiol., 72: 634-43, 1994.

25. Kabayashi J, Ohta M, Terado Y.C fiber generates a slow Na^+ spike in the frog sciatic nerve. Neurosci. Lett, 162: 93-6, 1993.

26. Quasthoff S, Grosskreutz J, Schroder JM, Schneider U, Grafe P. Calcium potentials and tetradotoxin resistant sodium potentials in unmyelinated C fibers of biopsied human sural nerve. Neuroscience, 69: 955-65, 1995.

27. Sah P. Different calcium channels are coupled to potassium channels with distinct physiological roles in vagal neurons. Proc. R. Soc. Lond. B. Biol. Sci., 260: 105-11, 1995.

28. Soliakov L, Wonnacott S. Voltage-sensitive Ca2+ channels involved in nicotinic receptor- mediated [3H] dopamine release from rat striatal synaptosomes. J. Neurochem., 67: 163-70, 1996.

29. Manca D, Ricard A.C, Trottier B, Chevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology, 67: 303-23, 1991.

30. Manca D, Ricard AC, Trottier B, Chevalier G. In vitro and in vivo responses of tissues to cadmium-induced lipid peroxidation. Bull. Environ. Contam. Toxicol., 46: 929-36, 1991.