

INHIBITORY EFFECT OF ALUMINIUM ON KCL AND PHENYLEPHRINE INDUCED CONTRACTION IN ISOLATED RAT AORTA

T. Mashhoodi^{*1}, S. Zahedi-Asl² and AR. Sarkaki²

1) Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

2) Department of Physiology, School of Medicine, Ahwaz University of Medical Sciences, Ahwaz, Iran

Abstract- It has been shown in some investigations that cardiovascular events are the main causes of death in hemodialysis patients. The exact etiology is unknown but some of the articles have reported a relation between aluminium ions in the dialysis solution and cardiovascular disorders. To determine the probable effect of aluminium on vasculature function, *in vitro* effects of aluminium ion on vasoconstriction induced by KCl (30 mM) or phenylephrine (10 μ M) were investigated using isolated rat aorta. AlCl₃ (1-4 mM) decreased both KCl and phenylephrine induced contractions in a dose dependent manner ($P < 0.01$). Complete inhibition of the contractions occurred using the higher doses of aluminium. Results of this study suggest that because of inhibitory effects of aluminium on vascular contractions, the probable cardiovascular dysfunction must be considered in aluminium intoxication. *Acta Medica Iranica*, 42(5): 379-382; 2004

Key words: Aluminium, phenylephrine, KCl, vasoconstriction, aorta, rat

INTRODUCTION

Aluminium, the third most abundant element in the earth's crust which is also used widely in industry and medicine, can be potentially toxic to human (1). Aluminium intoxication occurs specially in dialysis patients who take large amounts of aluminium via dialysis solution and consuming phosphate binders and is manifested mainly by encephalopathy (2), bone disease (3) and anemia (4). The role of aluminium in cardiovascular events which are the main causes of death in most of the hemodialysis patients (5) is not clear, but some evidence shows a relation between such disorders and aluminium intoxication (6-8). It has been shown that aluminium can accumulate in median layer of human arteries

(9). In the neuronal studies, it is shown that phosphoinositide signal transduction and voltage dependent calcium channels, both involved in vascular smooth muscle contraction (10), are disturbed by aluminium (11,12). In this study the effect of aluminium on contractility of isolated rat aorta was investigated.

MATERIALS AND METHODS

Male Wistar rats (280-310 g) were kept under standard conditions (12:12 hr cycle, 22 \pm 2 °C) and at the day of the experiment were killed by cervical dislocation. Thoracic aorta was removed and placed in Krebs solution with the following composition: (mM) NaCl: 118, KCl: 4.7, CaCl₂: 2.2.5, MgSO₄: 1.6, KH₂PO₄: 1.2, Glucose: 10 and NaHCO₃: 24. The aorta was carefully cleaned of adhering fat and connective tissues and then a ring of 4-6 mm in length, approximately equal to 4-6 mg, was isolated

Received: 7 Dec. 2002, Revised: 4 Nov. 2003, Accepted: 3 Mar. 2004

* Corresponding Author:

T. Mashhoodi, Department of Pharmacology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
Tel: +98 711 2330512
E-mail: T_mashhoodi@hotmail.com

and suspended between the bases of two triangular-shaped wires in an isolated tissue bath containing 20 ml of bubbled (95% O₂-5% CO₂) Krebs solution maintained at 37° C and pH of 7.3-7.4. One of the wires was attached to a fixed tissue support while the other was connected by a silk thread to an isometric transducer and the tension was recorded by a pen recorder (Harvard Universal Oscillograph). The tissue then was allowed to equilibrate for a period of 90 min while a tension of 2 gm was applied on it. The bathing solution was changed every 20 min and also before the addition of any agent.

The pharmacological agents used were KCl (Merck, Rahway, NJ) dissolved in distilled water and freshly used, phenylephrine hydrochloride (Sigma, St. Louis, MO) and AlCl₃. 6H₂O (Merck) dissolved in distilled water to give stock solutions of 10mM and 1M respectively and kept cold. The concentration of each drug is expressed as the final concentration in the organ chamber.

After the equilibration period several doses of phenylephrine and KCl administered to the tissue and a dose response curve was established. AlCl₃ (1-4 mM) was applied at the peak of the contractions induced by EC₅₀ of KCl (30 mM) and maximum dose of phenylephrine (10μM). Thus the EC₅₀ (the concentration in which the half of maximum effect is observed) concentrations of AlCl₃ were determined. In another series of experiments the maintenance of contractions induced by KCl and Phenylephrine after administration of distilled water was tested.

Because of the permanent effect of aluminium observed in pilot study, in all of the above experiments each tissue preparation was used for administration of one dose of AlCl₃.

The isometric contractile responses were expressed in terms of mg tension per mg tissue. Responses to aluminum were expressed as percentage of remained contractile response to total contraction induced by KCl or phenylephrine. The data were expressed as mean ± SEM. Statistical analysis between the responses to different concentrations of each drug was performed using analysis of variance and *P* values less than 0.05 were defined as significant.

RESULTS

KCl and phenylephrine could contract the tissue in a dose dependent manner which was not affected by using vehicle. AlCl₃ decreased both KCl and phenylephrine-induced contractions in a dose dependent manner (*P*<0.01) (Fig 1).

Aluminium (1.5 mM) inhibited 50% of 10μM phenylephrine induced contraction significantly. Dose of 4mM aluminium inhibited the contraction completely.

Aluminium with a concentration of 1.75 mM significantly inhibited 50% of 30mM KCl-induced contraction and a dose of 4 mM aluminium inhibited the contraction completely (Figures 2 and 3).

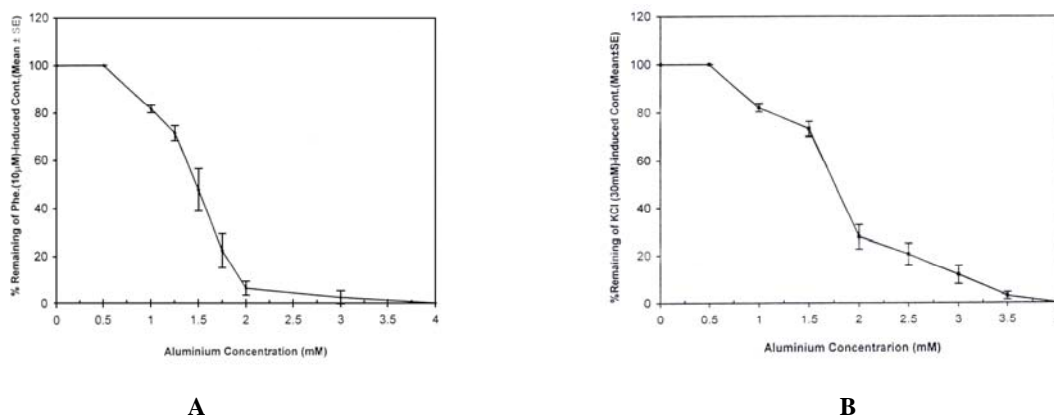


Fig 1. Percent contraction remained after administration of AlCl₃ to the aortic tissue, which contracted by phenylephrine (10μM) (A) and KCl (30mM) (B) ; *P*<0.01, n=7.

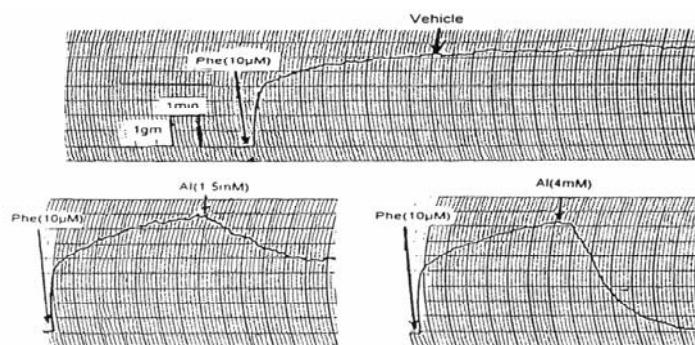


Fig 2. The inhibitory effect of AlCl_3 (1.5, 4 mM) on contraction induced by phenylephrine (10 μM)

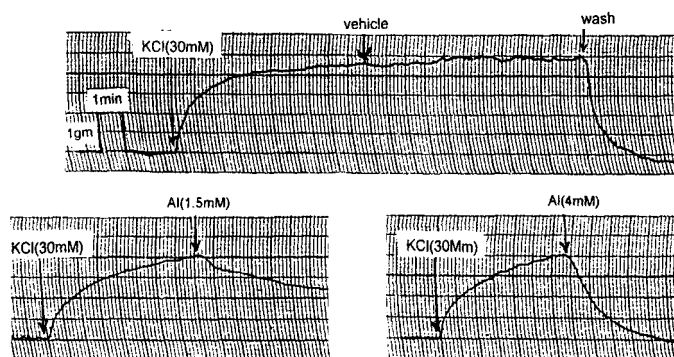


Fig 3. The inhibitory effect of AlCl_3 (1.5, 4 mM) on contraction induced by KCl (30 mM)

DISCUSSION

Aluminium inhibited the phenylephrine-induced contraction of aortic tissue. Although the exact mechanism(s) of aluminium function can not be established from the results of this study, it has been shown in other cells and especially neurons that aluminium interferes with phospho-inositol signal transduction in different ways (13-17). The inhibitory action of aluminium on the contractions induced by phenylephrine which is an α_1 -receptor agonist and activates phosphoinositide signal transduction (10, 18), may be accomplished by interfering with this mechanism.

Aluminium also inhibited the KCl-induced contraction of aortic tissue by mechanisms which again have not been established in this study. It is well known that KCl opens the voltage dependent calcium channels of smooth muscle by depolarizing

the cell membrane, so the tissue is contracted due to the rise of intracellular calcium (19). The inhibitory effect of aluminium on KCl induced contraction of aortic tissue may be due to blocking effect of this element on voltage dependent calcium channels which also has been demonstrated in the other cells such as rat dorsal root ganglion, rabbit atrial and ventricular cells and smooth muscle of rat colon (12, 20-24).

Ca-ATPase , protein kinase C and calmodulin are other biological systems which are known to be interfered with aluminium (25, 26) and they may be affected in this experiment too. Although proving all of the above possible mechanisms of the action of aluminium on vascular tissue needs more investigations, the results of this study suggest, for the first time, that aluminium intoxication may change vascular function and cause cardiovascular disorders.

REFERENCES

1. Habs H, Simon B, Thiedeman KU, Howe P. Aluminium. In: IPCS, Environmental Health Criteria 194. World Health Organization. Geneva 1997.
2. Boegman RJ, Bates LA. Neurotoxicity of aluminum. *Can J Physiol Pharmacol.* 1984 Aug;62(8):1010-1014.
3. Alfey AC. Aluminium intoxication. *N Engl J Med* 1984; 310: 1113-1115.
4. Rosenlof K, Fyhraust F, Tenhunen R. Erythropoietin, aluminium and anemia in patients on hemodialysis. *Lancet* 1990; 335: 247-249.
5. US RENAL DATA SYSTEM: 1992 Annual Report: IV. Comorbid conditions and correlations with mortality risk among 3,399 incident hemodialysis patients. *Am J Kidney Dis* 1992; 20 (suppl 2): 32-38.
6. Parfrey PS, Harnet J, Barre PE. The natural history of myocardial disease in dialysis patients. *J AM Soc Nephrol* Jul. 1991; 2 (1): 2-12.
7. London GM, Deverejoul MC, Fabiani F, Marchais S, Guerin A, Metivies P, Chapus P, Isach F. Association between aluminium accumulation and cardiac hypertrophy in hemodialysis patients. *Am J kidney Dis.* 1989; 13: 75-78.
8. Granadillo VA, Tahan JE, Salgado O, Ekjalde LE, Rodriguesiturb B, Romero GB, Romero RA. The influence of the blood levels of lead, aluminium and vanadium upon the arterial hypertension. *Clin Chim Acta* 1995; 233(1-2): 47-59.
9. Minami T, Ichii M, Tohno Y, Tohno S, Utsumi M, Yamada MO, Okazaki Y. Age-dependent aluminium accumulation in the human aorta and cerebral artery. *Biol Trace Elem Res.* 1996; 55(1-2): 199-205.
10. Scarborough NL, Garrier Co. Nifedipine and alpha adrenoceptors in rat aorta. 1. Role of extracellular calcium in alpha-1 and alpha-2 adrenoceptor- mediated contraction. *J Pharmacol Exper Therap.* 1984; 231 (3): 597-602.
11. Shafer TJ, Mundy WR, Tilson HA. Aluminium decreases muscarinic, adrenergic and metabotropic receptor-stimulated phosphoinositid hydrolysis in hippocampal and cortical slices from rat brain. *Brain Res* 1993; 624: 133-140.
12. Platt B, Busselberg D. Actions of aluminum on voltage-activated calcium channel currents. *Cell Mol Neurobiol.* 1994 Dec; 14(6): 819-829.
13. Shafer TJ, Mundy WR. Effects of aluminum on neuronal signal transduction: mechanisms underlying disruption of phosphoinositide hydrolysis. *Gen Pharmacol.* 1995 Sep;26(5):889-895.
14. Shafer TJ, Nostrandt AC, Tilson HA, Mundy WR. Mechanisms underlying AlCl₃ inhibition of agonist-stimulated inositol phosphate accumulation. Role of calcium, G-proteins, phospholipase C and protein kinase C. *Biochem Pharmacol.* 1994 Apr 20;47(8):1417-1425.
15. McDonald LJ, Mamrack MD. Aluminum affects phosphoinositide hydrolysis by phosphoinositidase C. *Biochem Biophys Res Commun.* 1988 Aug 30;155(1):203-208.
16. Shi B, Haug A. Aluminium interferes with signal transduction in neuroblastoma cells. *Pharmacol Toxicol.* 1992 Oct; 71(4): 308-313.
17. Jones DL, Kochian LV. Aluminum interaction with plasma membrane lipids and enzyme metal binding sites and its potential role in Al cytotoxicity. *FEBS Lett.* 1997 Jan 2;400(1):51-57.
18. Buckner SA, Oheim KW, Morse PA, Knepper SM, Hancock AA. Alpha 1-adrenoceptor-induced contractility in rat aorta is mediated by the alpha 1D subtype. *Eur J Pharmacol.* 1996 Feb 22;297(3):241-248.
19. Flaim SF. Comparative pharmacology of calcium blockers based on studies of vascular smooth muscle. In: Flaim SF, Zelis R. Calcium Blockers: Mechanisms of action and clinical applications. Baltimore: Urban and Schwarzenberg; 1982. p. 155-178.
20. Busselberg D. Calcium channels as target sites of heavy metals. *Toxicol Lett.* 1995 Dec;82-83:255-261.
21. Busselberg D, Platt B, Michael D, Carpenter DO, Haas HL. Mammalian voltage-activated calcium channel currents are blocked by Pb²⁺, Zn²⁺, and Al³⁺. *J Neurophysiol.* 1994 Apr; 71(4):1491-1497.
22. Busselberg D, Platt B, Haas HL, Carpenter DO. Voltage gated calcium channel currents of rat dorsal root ganglion (DRG) cells are blocked by Al³⁺. *Brain Res.* 1993 Sep 17; 622(1-2): 163-168.
23. Meiri H, Shimoni Y. Effects of aluminium on electrical and mechanical properties of frog atrial muscle. *Br J Pharmacol.* 1991 Feb;102 (2): 483-491.
24. Hava M, Hurwitz A. The effect of aluminum chloride on ⁴⁵Ca fluxes in isolated longitudinal smooth muscle from rat colon. *Arch Int Pharmacodyn Ther.* 1974 Nov;212(1):24-31.
25. Julka D, Gill KD. Altered calcium homeostasis: a possible mechanisms of aluminium-induced neurotoxicity. *Biochim Biophys Acta.* 1996 Jan 17;1315(1):47-54.
26. Siegel N, Haug A. Aluminum interaction with calmodulin. Evidence for altered structure and function from optical and enzymatic studies. *Biochim Biophys Acta.* 1983 Apr 14;744(1):36-45.