

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

T. Mashhoodi<sup>\*1</sup>, S. Zahedi-Asl<sup>2</sup> and AR. Sarkaki<sup>2</sup>

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  $\mu$ M) were investigated using isolated rat aorta. AICl<sub>3</sub> (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.

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**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  $^{\circ}$ 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, CaCl 2:2.5, MgSO<sub>4</sub>: 1.6, KH<sub>2</sub>PO<sub>4</sub>: 1.2, Glucose: 10 and NaHCO<sub>3</sub>: 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

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### \* 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

## Inhibitory effect of Aluminium on KCl and phenylephrine induced contraction

and suspended between the bases of two triangular-shaped wires in an isolated tissue bath containing 20 ml of bubbled (95% O<sub>2</sub>-5% CO<sub>2</sub>) 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 AlCl<sub>3</sub>·6H<sub>2</sub>O (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. AlCl<sub>3</sub> (1-4 mM) was applied at the peak of the contractions induced by EC<sub>50</sub> of KCl (30 mM) and maximum dose of phenylephrine (10μM). Thus the EC<sub>50</sub> (the concentration in which the half of maximum effect is observed) concentrations of AlCl<sub>3</sub> 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 AlCl<sub>3</sub>.

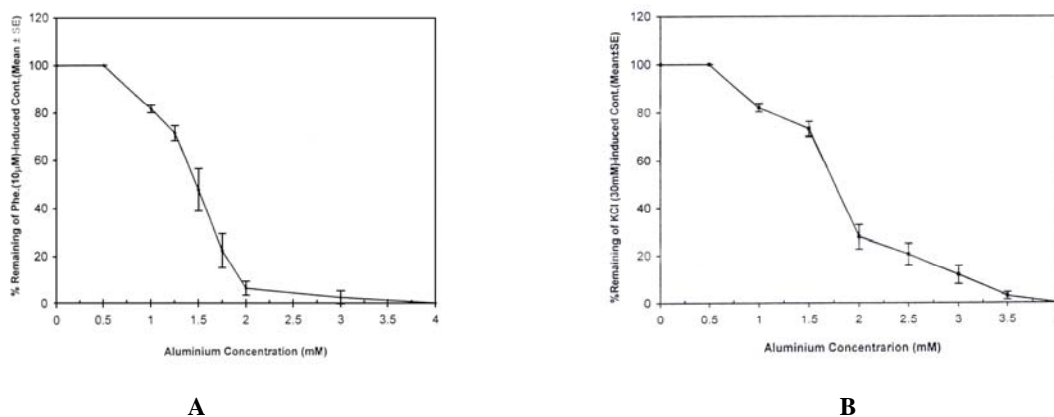
The isometric contractile responses were expressed in terms of mg tension per mg tissue. Responses to aluminium were expressed as percentage of remained contractile response to total contraction induced by KCl or phenylephrine. The data were expressed as mean ± SEM. Statistical analysis between the responses 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. AlCl<sub>3</sub> 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 AlCl<sub>3</sub> to the aortic tissue, which contracted by phenylephrine (10μM) (A) and KCl (30mM) (B) ; *P*<0.01, n=7.

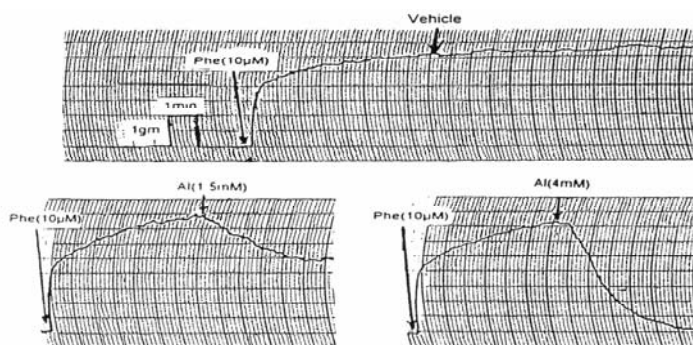


Fig 2. The inhibitory effect of  $\text{AlCl}_3$  (1.5, 4 mM) on contraction induced by phenylephrine (10  $\mu\text{M}$ )

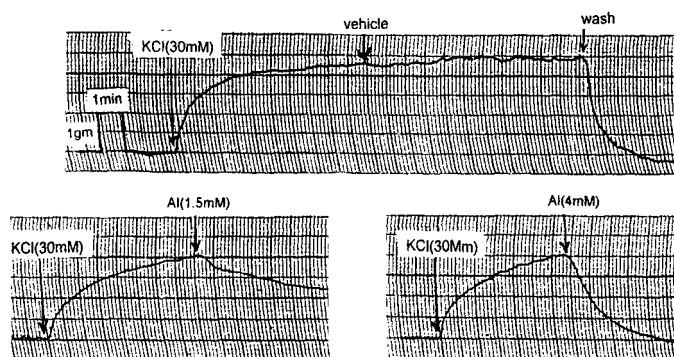


Fig 3. The inhibitory effect of  $\text{AlCl}_3$  (1.5, 4 mM) on contraction induced by KCl (30 mM)

## 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  $\alpha_1$ -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).

$\text{Ca-ATPase}$ , 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.

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