The Clot-lysis Effect of Selective α_1-Adrenoceptor Antagonist in Vitro Model Associated with High Peroxynitrite Level

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ABSTRACT:

BACKGROUND:

Alpha$_1$-adrenoceptor blocking agents showed several effects beyond their action on the vascular smooth muscles. They improve the lipid profile and inhibit the aggregation of blood platelets.

OBJECTIVE:

To investigate the clot-lysis effect of selective α_1-adrenoceptor antagonists and its relation to peroxynitrite level *in vitro* experimental model.

MATERIALS AND METHODS:

Venous blood samples obtained from ten healthy subjects. To each pre-weighed clot, 100 µL of either distilled water as a negative control, prazosin (10 µg), terazosin (20 µg) and alfuzosin (25 µg) were added. Peroxynitrite level was measured in sera and sangious fluid that formed after clot-lysis.

RESULTS:

Prazosin, terazosin and alfuzosin,in order, significantly reduced the clot weight up to 3.7%. Peroxynitrite level in sangious fluids was higher in treated groups than that of negative control or sera levels.

CONCLUSION:

α_1-adrenoceptor antagonists induced clot-lysis effect. This effect is associated with generation peroxynitrite

KEY WORDS: clot-lysis, α_1-adrenoceptor antagonist, peroxynitrite

INTRODUCTION:

Selective α_1-adrenoceptor antagonists caused smooth muscle relaxation in peripheral vasculature in arterial and venous dilation[(1)]. As well, they relaxed smooth muscle of the bladder neck and prostate [(2)]. They are indicated for hypertension [(3)] and for the symptomatic treatment of benign prostate hypertrophy [(4)].

Prazosin, *in vivo*, had no effect on platelet mediated thrombosis [(5)] while *in vitro* reduced thrombus formation when it combined with plasmin[(6)].

Doxazosin treatment in patients with essential hypertension resulted in increase of fibrinolytic potential via increase in tissue-plasminogen activator (t-PA) mass concentration [(7)] and decrease in plasma plasminogen activator inhibitor (PAI) [(8)].

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Terazosin improved the prothrombotic state of patients with essential hypertension as a result of significant increase nitric oxide (NO) [(9)]. Nielsen *et a*l reported that peroxynitrite ($ONOO^-$), an end-product of NO, inhibits t-PA resulting in inhibition of fibrinolytic activity [(10)].

This study aimed to investigate the clot-lysis effect of selective α_1-adrenoceptor antagonists in reference to the peroxynitrite level *in vitro* experimental model

MATERIALS AND METHODS:

This work was done at Department of Pharmacology, College of Medicine, Al-Mustansiriyia University in Baghdad, Iraq during December 2009. The study was approved by the scientific committee of institute, and after obtaining permission from the local ethics committee and informed subject consent, subjects from healthy medical students were allocated randomly to enroll in the study.

Venous blood samples (3 milliliters) were drawn from ten healthy human medical students. 500 µl of blood was transferred to each four previously weighed eppendorff tubes for each subject.

The transferred 500 µL allowed to clotting at 37ºC for 60 minutes [11]. After clot formation, serum was completely removed and kept for determination of peroxynitrite level. Each tube with clot was again weighed to determine clot weight (clot weight (mg)= weight of tube containing clot minus weight of tube alone).. To each eppendorff contained pre-weighed clot, 100 µL of either distilled water as a negative control, prazosin HCl (10 µg), terazosin HCl (20 µg) and alfuzosin HCl (25 µg) were added. All the tubes were then incubated at 37ºC for 90 minutes and observed for clot lysis. After incubation, the released sangious fluid of each treatment was removed, pooled, centrifuged and the supernatant kept for determination peroxynitrite level. The tubes were again weighed. The difference in weight was expressed as percentage of stabled or lysed clot.

Peroxynitrite level in pooled sera (before treatment) and pooled sangious fluid (after each treatment) were determined according to the method described by Beckman *et al*[12]cited by VanUffelen *et al* [13] . Peroxynitrite mediated nitration of phenol resulting in nitrophenol formation, formed the basis of peroxynitrite assay.

Drugs and chemicals

All the chemicals used in the study were of analar grade. Prasozin HCl , Terazozin HCl (Sigma-Aldrich, St Louis, Missouri) and Alfuzosin HCl (Safoni-Synthelabo, France) were freshly prepared according to the manufacturer instructions.

Statistical analysis

Data were expressed as mean ± SD of observations (n=10). The significance was $p \leq 0.05$ between percent changes in clot weight induced by each treatment tested by ANOVA test.

RESULTS:

Table 1 showed that α_1-adrenoceptor antagonists significantly induced clot lysis as compared with negative control. Although the effect of prazosin HCl is more than terazosin HCl and alfuzosin HCl, it did not reach to the level of significant. It is. The mean level of peroxynitrite in sera is higher than that of sangious fluid of negative control (5.681 µmol *vs* 4.545 µmol). Peroxynitrite level of sangious fluids belonged to α_1-adrenoceptor antagonists induced clot-lysis was higher than that of negative control and sera level (Fig.1). The increment in peroxynitrite level is inversely proportional to the percent of clot lysis. The more clot-lysis effect , the lesser peroxynitrite level in respect to α_1-adrenoceptor antagonist.

Table 1: Effect α_1-adrenoceptor antagonists on blood clot.

Treated groups	Clot weight(mg) before treatment	Clot weight (mg) after treatment	% change
Distilled water	483.2 ± 22.66	476.86 ± 22.06	-1.374 ± 0.551
Prazosin (10 µg)	518.9 ± 20.49	499.71 ± 17.8	-3.676 ± 1.316**
Terazosin (20 µg)	495.91 ± 34.43	480.21 ± 29.98	-3.166 ± 1.446**
Alfuzosin (25 µg)	498.3 ± 32.26	487.54 ± 32.22	-2.109 ± 0.754*

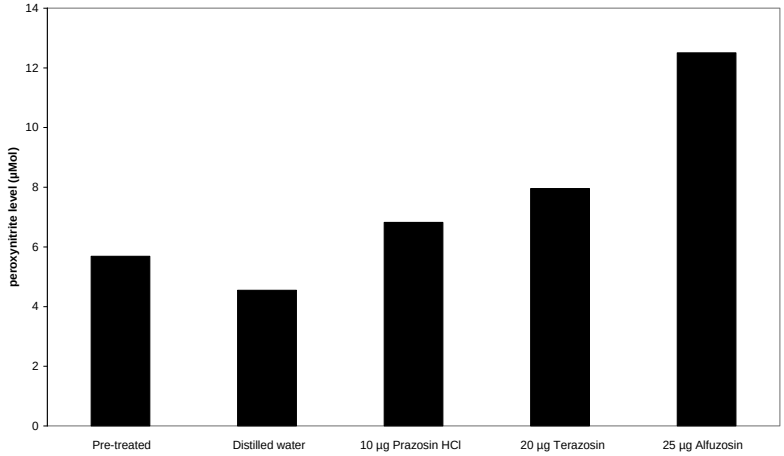
* $p < 0.05$, ** $p < 0.01$: in comparison with distilled water treated group.

DISCUSSION:

This study shows that α_1-adrenoceptor antagonists lysed clot from the blood of healthy individuals. Prazosin HCl, terazosin HCl and alfuzosin HCl that are used for benign prostatic hypertrophy were not reported to have antithrombotic or fibrinolytic effect. The other intriguing finding is the higher mean level of $ONOO^-$ in sangious fluids, of clot lysed by α_1-adrenoceptor antagonists, than corresponding negative control or sera.

There is cumulative evidence that $ONOO^-$ attacked tissue factor and inhibits procoagulant activity [14], and it inhibits fibrinogen activity (IC_{50} is 22 µmol)leading to inhibit clot formation [15]. Moreover, nitronyl nitroxide containing peptides possessed thrombolytic activity [16] and singlet oxygen radical potentiates thrombolysis induced by polymorphnuclear neutrophils [17].

Therefore, the clot-lysis effect of α_1-adrenoceptor antagonists may be related to their effect on generation nitrogen species [18,19] by the evidence of high $ONOO^-$ that formed from interaction of nitric oxide and superoxide anion.



The mean level of peroxyritrite insere (pre-treated)and sangiuos fluid(post-treated)

CONCLUSION:

On the minor clot-lysis effect in vitro, α_1-adrenoceptor antagonists may be incorporated for improvement of patients suffering from thrombotic disorders. Further investigation is essential to elucidate their mechanism of action.

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