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# THROMBOLYTIC EFFECT OF SOME ANTIBIOTIC DRUGS: *IN VITRO* AND *IN SILICO* APPROACH.

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## ABSTRACT

The present study aims to investigate the thrombolytic effect of some antibiotic drugs by *in vitro* clot lysis method and *in silico* molecular docking used to identify whether these drugs interact with the responsible protein (tissue-type plasminogen activator). *In vitro* clot lysis model was used to observe the thrombolytic effect of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs provided the clot lysis 32.03±1.78%, 38.25±3.41%, 33.49±2.03%, 36.96±2.47%, 33.94±4.2%, 34.32±1.77% and 40.41±2.15% clot lysis, respectively. Reference drug streptokinase exhibited 78.70±0.92% clot lysis. A wide range of docking score found during molecular docking by CPI server. Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs showed the docking score -8.0, -7.3, -7.2, -7.2, -6.8, -7.4 and -9.1, respectively. Rifampin possessed highest clot lysis effect and also showed best docking score among the antibiotics. Further *in vivo* investigation need to identify the thrombolytic effect of these antibiotics and also require making out the mechanism of them as thrombolytic agents.

**KEYWORDS:** Antibiotic drugs, Molecular docking, Amoxicillin, Ciprofloxacin, Rifampin.

## 1. INTRODUCTION

Thromboembolic diseases are serious and life threatening. Despite the availability of antithrombotic drugs for the prevention and treatment of arterial and venous thrombosis, thrombotic diseases continue to be a major cause of death and disability worldwide. Therefore, there remains a need for more effective therapies to combat these disorders.[1]

Molecular docking is a computational chemistry method which has become essential for the rational drug design process.[2-4] Molecular docking has become a major computational method for the prediction of ligand–receptor interactions.[5] Over the previous couple of years the amount of latest molecular targets has increased because of the completion of the human genome project, also because the protein and protein–ligand complex structures isolated by high-throughput protein purification[6] and solved by crystallography and nuclear magnetic resonance spectroscopy techniques.[7,8] At an equivalent time, the advance of computational techniques for finding out interactions of ligands with the biological targets at the atomic scale have increased and developed.[9,10]

Our aim of this study to investigate the thrombolytic effect of some antibiotic drugs like, Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin, which were studied by using *in vitro* clot lysis and *in silico* Molecular docking model. However, no earlier studies have been conducted experimentally to characterize the thrombolytic effect of these antibiotics.

## 2. MATERIALS AND METHODS

### 2.1 *In vitro* thrombolytic effect

#### 2.1.1 Drugs and chemicals

Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin were got from a renowned Pharmaceuticals company as gift, Bangladesh. To the commercially available lyophilized streptokinase (SK) vial (Square Pharmaceuticals Ltd.) of 1500000 I.U., 5mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 $\mu$L (30,000 I.U.) was used for *in vitro* thrombolysis. All chemicals and reagents were of reagent grade.

#### 2.1.2 Drugs solution preparation

A 100 mg each of the drugs was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to

remove the soluble supernatant, which was filtered through a 0.22-µm syringe filter. A 100 µl of this aqueous preparation was added to the Eppendorf tube tubes containing the clots to check thrombolytic activity.

### 2.1.3 *In vitro* Thrombolytic effect assay

Experiments for clot lysis were carried as reported earlier.[11] Briefly, 4.5 ml venous blood drawn from the healthy volunteers was distributed in nine different pre weighed sterile Eppendorf tube (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each Eppendorf tube containing pre-weighed clot, 100µl of aqueous solution of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin were added separately. As a positive control, 100 µl of SK and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated with the blood samples of the 12 volunteers.

### 2.2 *In silico* Molecular docking

All the antibiotic drugs structure need to upload in mol2 format with charges and hydrogens added. When a molecule submitted, The CPI server checks the format suitability and calculates the interaction profile of this drug towards all the targets in the database using DOCK6.[12,13] Users can view the real-time progress online and the page showing the current docking status of the uploaded drug will also be provided for bookmarking.[4,14] It takes between 6 and 20 h to finish a one-molecule task and an email will be sent on completion. The outputs comprise the two following major elements:

(i) Library drugs which share similar (or opposite) interaction profile with the user's molecule, ranked by the similarity (or disparity) with known indications and ADR information, suggesting the underlying new indication and ADR of the user's molecule.[15]
(ii) The candidate off-targets that tend to interact with the user's molecule. The server will visualize the drug-protein interactions, with amino acid residues around 6A$^{o}$ of the molecule highlighted.[16]

### 2.3 Statistical analysis

The significance between % clot lysis by SK and drugs tested by Tukey test using the software SPSS, version 22.0 (SPSS for Windows, Version 22.0, IBM Corporation, New York, USA). Data are expressed as mean ± SEM. The mean difference between positive and negative control was considered significant at *P* values < 0.05 and 0.0001.

## 3. RESULTS

### 3.1. *In Vitro* Thrombolytic effect

In thrombolytic effect assay, addition of 100µl streptokinase as positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37°C, showed 79.50±1.18% lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (5.70±1.80%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant (P<0.0001). Treatment of clots with Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs provided the clot lysis 32.03±1.78%, 38.25±3.41%, 33.49±2.03%, 36.96±2.47%, 33.94±4.2%, 34.32±1.77% and 40.41±2.15%, respectively. All the results presented in Table 1 and Figure 1.

**Table 1: Clot lysis effect of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin**

| drugs | % of clot lysis (mean ± SEM) |
|---|---|
| Negative control (water) | 5.70±1.80 |
| Positive control (streptokinase) | 79.50±1.18 [a] |
| Amoxicillin | 32.03±1.78[a,b] |
| Azithromycin | 38.25±3.41 [a,b] |
| Aztreonam | 33.49±2.03 [a,b] |
| Cephalospirin | 36.96±2.47 [a,b] |
| Ciprofloxacin | 33.94±4.2 [a,b] |
| Phenoxymethylpenicillin | 34.32±1.77 [a,b] |
| Rifampin | 40.41±2.15 [a,b] |

**Drugs on human blood**

Values are mean ± SEM ($n$ = 12); [a]$P$ <0.0001, Tukey test as compared to negative control, [b]$P$ < 0.001, compared to positive control. Statistical representation of the effective clot lysis percentage by drugs preparations, positive thrombolytic control (streptokinase) and negative control (sterile distilled water) processed by Tukey test by using SPSS for windows, version 22.0.

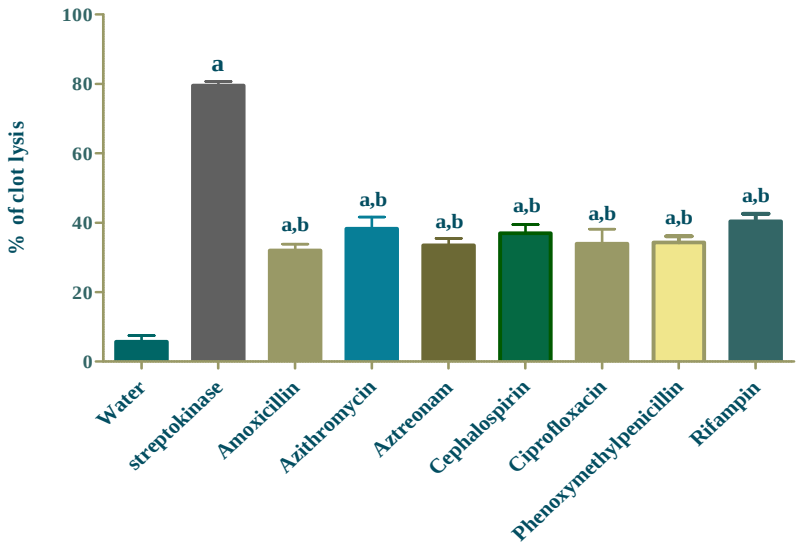


**Figure 1: Clot lysis effect of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs on human blood.**

Values are mean ± SEM ($n$ = 12); [a]$P$ <0.0001, Tukey test as compared to negative control (Water), [b]$P$ < 0.001, compared to positive control (Streptokinase). Statistical representation of the effective clot lysis percentage by drugs preparations, positive thrombolytic control (streptokinase) and negative control (sterile distilled water) processed by Tukey test by using SPSS for windows, version 22.0.

### 3.2 *In silico* Molecular docking

In the present study, molecular docking performed to identify the docking score of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin towards tissue-type plasminogen activator (PDB code 1A5H), which is a protein involved in the breakdown of blood clots. A wide range of docking score found during molecular docking by CPI server. Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs showed the docking score -8.0, -7.3, -7.2, -7.2, -6.8, -7.4 and -9.1, respectively. All the results presented in Table 2 and Figure 2.

**Table 2: Docking results with selected antibiotic in the tissue-type plasminogen activator.**

| Drug name | Docking Score |
|---|---|
| Amoxicillin | -8.0 |
| Azithromycin | -7.3 |
| Aztreonam | -7.2 |

| | |
|---|---|
| Cephalospirin | -7.2 |
| Ciprofloxacin | -6.8 |
| Phenoxymethylpenicillin | -7.4 |
| Rifampin | -9.1 |

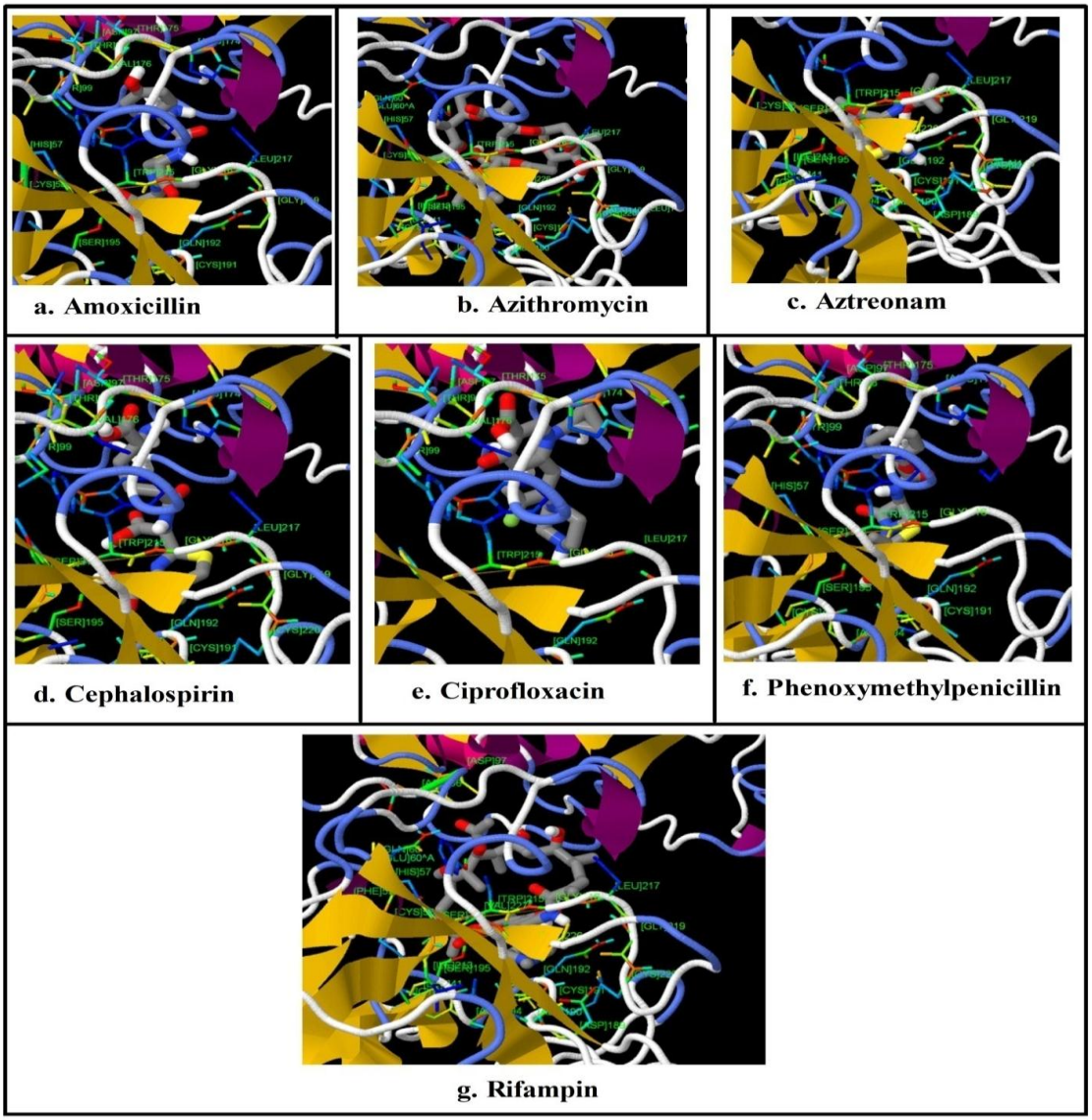


**Figure 2: Molecular docking analysis of Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin with tissue-type plasminogen activator complex obtained from docking.**

## 4 DISCUSSIONS

In our thrombolytic assay, the comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. When compared with the clot lysis percentage obtained through water, a well significant (*P* value < 0.001) thrombolytic activity was observed after treating the clots Amoxicillin, Azithromycin,

Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin. However, the clot lysis value for Rifampin was higher than other drugs.

In molecular docking study, Amoxicillin, Azithromycin, Aztreonam, Cephalospirin, Ciprofloxacin, Phenoxymethylpenicillin and Rifampin drugs showed the docking score -8.0, -7.3, -7.2, -7.2, -6.8, -7.4 and -9.1, respectively towards tissue-type plasminogen activator. From all these antibiotic drugs, Rifampin exhibited best docking score (-9.1), which also possessed maximum clot lysis (40.41±2.15%) effect on human blood. After Rifampin, Azithromycin showed low clot lysis (35.89 ± 3.57%) effect compare to standard drugs (streptokinase) and also exhibited well docking score (-7.3). On the other hand, Amoxicillin showed lowest clot lysis (32.03±1.78) effect among the tested drugs.

From the present study, it was clear that antibiotic drugs have moderate to well thrombolytic effect. We also found that some of them also have good thrombolytic effect and also they showed well docking score for tissue-type plasminogen activator, so we can think the use of antibiotic drugs for thrombosis management. However further investigation need to proof the thrombolytic effect of these antibiotic drugs in *in vivo* model.

## 4. CONCLUSION

Rifampin possessed highest clot lysis effect and also showed best docking score among the antibiotic drugs. Further *in vivo* investigation need to identify the thrombolytic effect of these antibiotic drugs and also require making out the mechanism of them as thrombolytic agents.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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