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THE EFFECTS OF AMIKACIN ON RED BLOOD CELL MEMBRANE STABILITY

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ABSTRACT

Background: Physicians widely use Amikacin as an aminoglycoside antibiotic to treat severe gram-negative infections. However, researchers must further evaluate its interaction with non-targeted cells, such as erythrocytes. **Objective:** To evaluate the impact of Amikacin on erythrocytes membrane integrity and hemolysis under controlled *in vitro* conditions. **Materials and Methods:** Washed human red blood cells were treated with increasing doses of Amikacin (25–200 µL/mL). Samples were maintained at 37 °C for 30 minutes, and red blood cells lysis was measured by spectrophotometer at 540 nm. Structural changes were evaluated microscopically. **Results:** Erythrocytes destruction enhanced in a dose-related manner, with negligible impact at lower concentrations and considerable cell destruction at higher concentrations. Microscopy confirmed progressive structural alterations in red blood cells. **Conclusion:** The disruption of erythrocytes at high doses of Amikacin, highlighting the importance to evaluate the drug - cell reaction.

KEYWORDS: Amikacin, Red blood cells, Hemolysis, Membrane integrity, Aminoglycosides, Infections.

INTRODUCTION

Antibiotics are important in clinical medicines to treat infections, but research is still under progress that how these drugs may affect the cells other than the intended target cells like red blood cells (RBCs). RBCs play an important role in oxygen transport and any damage to membrane integrity may lead to medical effects due to loss of physiological homeostasis.[1,2]

Aminoglycosides are potent, broad-spectrum antibiotics that act through inhibition of protein synthesis. However, the drug may interact with cellular surfaces and disrupt the membrane integrity under conditions including high concentration of drug, long- term exposure, or membrane with increased components of negative charges.[3,4] RBCs provide a well-accepted model for investigating the drug- induced alterations in the membrane structure.[5]

Amikacin is a semi-synthetic aminoglycoside, used to treat severe, resistant bacterial infections. It has well established antibacterial activity against a wide variety of Gram-negative organisms.[6,7] However, it's potential effects on RBCs inducing osmotic fragility in dose - dependent manner requires further investigation.[8]

Among certain factors, one of the factor responsible for destruction of RBCs is the drug – induced hemolysis. This drug – dependent increased membrane fragility is associated with increased oxidative stress, either by generating reactive oxygen species or decreasing cellular antioxidant activity.[9,10] The evaluation of hemolytic activity under controlled laboratory conditions is crucial for the analysis of safety characteristics of the drug.

Therefore, this study aimed to evaluate the effect of Amikacin on RBC membrane stability and hemolysis under *in vitro* conditions.

MATERIALS AND METHODS

Experimental Setup

Six treatment groups were established with Amikacin concentrations of 25, 50, 75, 100, 150, and 200 µL / mL. Each concentration was tested in replicates to ensure

reliability.

Blood Sampling and Preparation
Blood was drawn from healthy volunteers in EDTA tubes adhering to ethical standards and approved procedures. Erythrocytes were separated by centrifugation, washed with isotonic saline, and re-suspended to the desired concentration.[11]

Drug Treatment
Amikacin was added to erythrocyes suspensions at the specified concentrations. A negative control (isotonic saline) and a positive control (distilled water) were included.

Incubation
Samples were incubated at 37 °C for 30 minutes.

Spectrophotometric Analysis
Post- incubation, samples were centrifuged and the supernatants were analyzed at 540 nm to quantify hemolytic activity.[12]
The experiments were performed with five different samples independently to ensure reproducibility, and results are expressed as mean ± SD.

Microscopic study
Peripheral blood smears were prepared, and observed under microscope to evaluate cellular morphological alterations.

Hemolysis Calculation
Hemolysis (%) was calculated by the formula:
Hemolysis Rate (%) = (A test – A negative control) / (A positive control – negative control) × 100
Where, A = Absorbance

RESULTS
Spectrophotometric Findings
Hemolysis increased progressively with increasing concentrations of Amikacin. Lower concentrations (25–50 µL/mL) produced minimal hemolysis, whereas higher concentrations (150–200 µL/mL) resulted in marked RBC membrane damage. The data demonstrated a clear concentration-dependent trend.

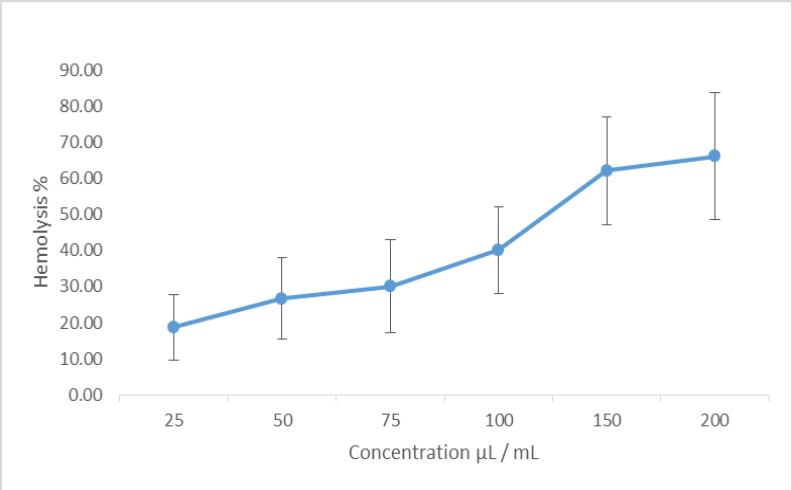

Fig. 1: Concentration-dependent hemolytic activity of Amikacin on human RBCs*
***Each value is the mean ± SD of 5 samples.**

Fig. 1 represented the effect of different concentrations of Amikacin on RBCs hemolysis. The hemolysis increased gradually with increasing doses, demonstrating a concentration - dependent trend. The lower doses (25–50 µL/mL) produced minimal hemolysis, whereas, higher doses (150–200 µL/mL) resulted in a significant destruction to RBCs membrane.

Microscopic Observations
Fig. 2a – 2g represented the drug – induced morphological variations in erythrocytes. The RBCs placed in normal saline i.e., in negative control, appeared as biconcave (Fig.2a), whereas, the RBCs treated with higher concentrations of antibiotic (100, 150, and 200 µL / mL) showed membrane deformation, cellular swelling and disintegration of RBCs membranes (Fig. 2e, 2f, 2g).

Fig.2a: Isotonic Saline

Fig.2b: 25 µL/mL Amikacin

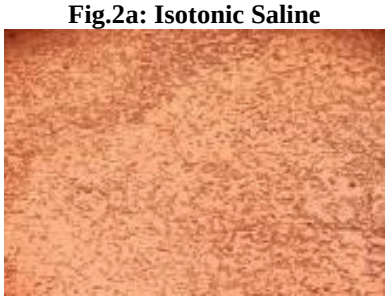

Fig. 2c: 50 µL/mL Amikacin

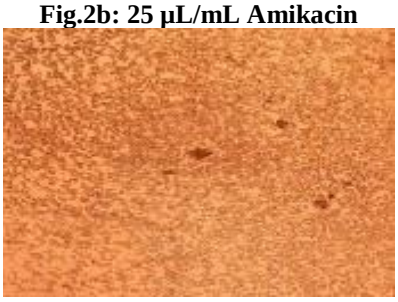

Fig. 2d: 75 µL/mL Amikacin

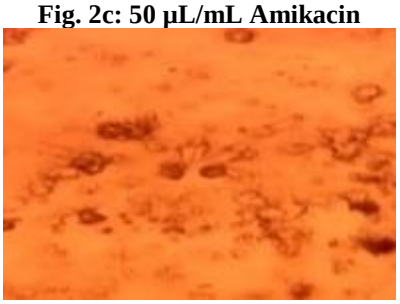

Fig. 2e: 100 µL/mL Amikacin

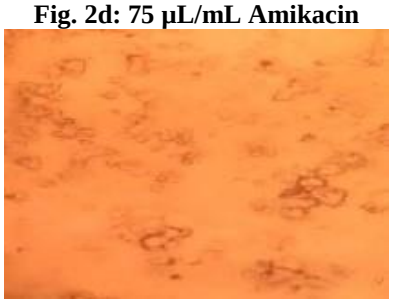

Fig. 2f: 150 µL/mL Amikacin

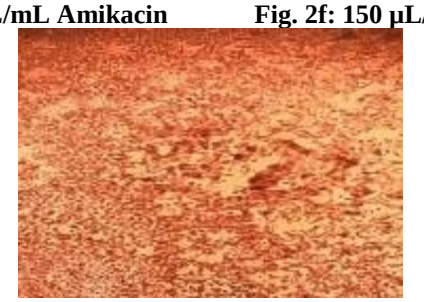

Fig. 2g: 200 µL/mL Amikacin.

Fig. 2: Drug- induced morphological alterations in erythrocytes.

DISCUSSION

The increased destruction of RBCs, observed in the present study indicate the capability of Amikacin to disrupt the RBCs membrane stability at elevated doses. This effect may be attributed to interplay with cellular surface constituents, leading to structural fragility.[13,14]

The visible morphological changes in the RBCs structure are supported by the spectrophotometric results (Fig.1), confirming the gradual changes and damage to the erythrocyte membrane. These results are in consistence with the previous study where selected antibiotics interacted with RBCs membrane leading to morphological alterations.[15]

Redox imbalance and increased lipid peroxidation are the well- established mechanisms involved in the drug-induced immune hemolytic anemia, responsible for oxidative injury to erythrocytes components.[9,10] The findings of the present study indicated that Amikacin is capable to induce both structural and functional impairments resulting in hemolysis.[14,16]

Moreover, the current studies suggested that hemolysis is strongly associated with decreased membrane flexibility and increased oxidative injury at cellular level.[17] Additionally, the emerging RBC - based drug interaction research highlights the crucial role of membrane stability in determining the response of RBCs to pharmacological agents.[18]

Erythrocytes serve as a sensitive model for studying foundational mechanism effects, and the present results reinforce the importance of evaluating this membrane targeting effect for systemic antibacterials.[5]

CONCLUSION

Amikacin affects erythrocyte integrity in a dose-related

manner, with elevated amounts causing substantial RBCs destruction and physical damage. These findings highlight the requirement for further studies to better comprehend the pathways underlying drug- related membrane changes.

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