Virological and Molecular Studies on Rotavirus

Ahmed Abdel Samie H. Ali, Ali A. Salama, Sayed M. Zeidan*, Shimaa M.G. Mansour

Department of Virology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt
* Rinderpest like Diseases Dept., Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo

ABSTRACT

Bovine Rotavirus (BRV) is a major cause of acute gastroenteritis in neonatal calf diarrhea throughout the world causing economic losses to beef and dairy industries. Neomycin B, an aminoglycoside antibiotic has been shown to inhibit human immunodeficiency virus and Herpes simplex virus replication. The current study was carried out to assess the effect of neomycin B on rotavirus replication in Rhesus monkey kidney fetal cells (MA-104) by immunofluorescence (IF), plaque assay and flow cytometry. Neomycin interfered with the development of BRV cytopathic effect (CPE). Neomycin concentrations at 5, 7.5 and 10 mM were able to reduce the intracytoplasmic fluorescence induced by BRV by16.67%, 41.67% and 83.33% respectively. There was marked decrease in the yield of viral RNA. Neomycin concentrations of 5, 7.5 and 10mM were observed to be able to reduce the percentage of cell infectivity with BRV by 16.21%, 56.72% and 73.94% respectively. The kinetics of BRV growth was carried out by plaque assay. At the concentrations 7.5 and 10mM neomycin, there was marked decrease in virus titer in supernatant at time interval 48 and 72h post infection. It was concluded that Neomycin B is an inhibitor of BRV replication *in-vitro* particularly when the drug is added during the complete infection cycle; pre-incubation of virus and pretreatment of MA-104 with neomycin does not affect replication of BRV.

INTRODUCTION

Bovine Rotavirus is classified within Genus *Rotaviruses* within the Family *Reoviridae*. The virus particle is triple layered, approximately 70 nm in diameter, and of icosahedral symmetry. Its genome consists of 11 segments of double-stranded RNA that code for 6 structural and 6 non-structural proteins. It also contains its RNA polymerase *(1)*. Infection with rotavirus occurs world-wide causing economic losses among calves under 3 weeks of age with morbidity and mortality rates reach 37% and 25 to 46% respectively *(2,3)*. Although rotavirus vaccines are often administered to pregnant animals to increase levels of rotavirus antibodies in colostrum, neonates frequently exhibit rotaviral diarrhea. The later may be attributed to failure of passive transfer from dam to calf *(4)*. Therefore other therapeutics need to be considered for controlling of Rotavirus infection.

A family of aminoglycoside antibiotics, including neomycin B, is capable of binding to RNA molecules *(5)* and targets the 30S subunit of ribosomal RNA causing mistranslation *(6)*. Neomycin B has been shown to inhibit viral replication by several mechanisms, including blockage of viral penetration or inhibition of viral nucleic acid synthesis *(7)*. Neomycin B inhibits the replication of herpes simplex virus type I in BHK cells by more than 90%, by binding to the virus receptor *(8)*. It interrupts the interaction between a protein and specific regions of the genome of human immunodeficiency viruses (HIV) *(9)*. The assumptions were based on previous results obtained with effect of neomycin B on other viruses. Our goal was to study the effect of neomycin on Rotavirus replication.

MATERIAL AND METHODS

Cell: MA-104 cell cultures (Rhesus monkey kidney fetal cells) were obtained from American Type Culture Collection (ATCC).

Bqvine rotavirus (BRV): Nebraska calf diarrhea virus (NCDV) was obtained from Department of Veterinary Sciences, South Dakota State University (SDSU), USA. This virus suspension was used throughout the experiment at a multiplicity of infection of 4000 PFU/ 10^6 cells.

Neomycin B (Sigma, USA) was prepared as a stock solution at 100 mM in MEM pH 7.3, sterilized by filtration and maintained at 4°C.

Antibody: Anti-NCDV VP6 monoclonal antibody prepared in mice and labeled with fluorescin isothiocyanate (FITC), obtained from Department of Veterinary Sciences, SDSU, USA. It used in IF and flow cytometry.

Preliminary assessment of Neomycin B cellular cytotoxicity: Confluent MA-104 cells grown in 96-well plates were washed and maintained with neomycin at the concentrations of 0.6, 3, 5, 7.5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM in MEM. Each concentration was tested in 6 wells per plate, in addition to mock-treated cells. The plate was incubated at 37°C in 5% CO2 condition for 4 days with daily examination for any changes indicating cytotoxicity ***(10).*** The cell viability was evaluated by a standard colorimetric assay for measuring cellular proliferation using 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Five hours before the end of incubation, 20 µl of MTT solution (5mg/ml in PBS) was added to each well containing cells. The plate was incubated for 5 hours; media was removed with needle and syringe. Two hundred µl of DMSO was added to each well to dissolve formazan crystals. The plate was read at 570 nm wavelength ***(11).*** The cytotoxic index (CI) was calculated by following equation:

$$CI = \frac{OD\ control - OD\ sample}{OD\ control} \times 100$$

Simultaneous treatment assay: Confluent MA-104 cells grown in 24-well plates were infected in quadruplicate with BRV and treated with different non-cytotoxic concentration (below CC_{50}), 0.6, 3, 5, 7.5 and 10 mM of neomycin in 1 x MEM containing 2.5 µg/ ml trypsin at the same time of infection. Monolayers treated and non-infected, monolayer non-treated and infected and monolayers non-treated and non-infected were maintained for control. The cells were incubated for 72 hours at 37°C in 5% CO2 condition with daily observation for the development of specific CPE ***(12).***

Treatment of BRV with neomycin B: Prior to inoculation, virus suspension was treated with 0.6, 3, 5, 7.5 and 10 mM of neomycin for 30 minutes at 37°C. Confluent MA-104 cells grown in 24-well plates were infected in quadruplicate with virus-neomycin suspension in addition to control wells. The inoculum excess was removed; cells were washed and maintained with fresh 1 x MEM with 2.5 µg/ml trypsin, and daily observed for 3 days ***(10).***

Pre-infection treatment assay: Treatment of MA-104 with neomycin before the infection: Confluent MA-104 cells grown in 24 wells plates were treated for one, 16 and 24 hours with 0.6, 3, 5, 7.5 and 10 mM of neomycin at 37°C, in quadruplicate. The neomycin excess was removed and monolayers were inoculated with BRV in addition to control wells. Following attachment, virus inocula were also removed and cultures were washed and handled ***(10).***

Treatment of cells with neomycin post-infection with BRV: Neomycin was added to the cells after virus adsorption time and then maintained and handled ***(12).***

Evaluation of Effect of neomycin B on BRV replication using Immunofluorescence: After infection period, MA-104 cells were fixed with 80% acetone for 20 minutes, incubated for one hour at 37°C with rotavirus VP6 MAb labeled with FITC, diluted 1/100 in PBS. The plates were washed 3 times with PBS then examined for specific positive intracytoplasmic fluorescence using Nikon Eclipse, TS100 Japan Fluorescent microscope ***(13).***

Evaluation of effect of neomycin B on BRV replication using plaque assay: Confluent MA-104 cells were infected with BRV. After

adsorption, the inoculum was removed and the cells were overlaid with media containing 0.8% seaplaque agarose and different concentration of neomycin B supplemented with 5ug/ml trypsin in addition to neomycin and cell controls. The cells were incubated for 5 days and then examined by fixation with 10% formol saline and staining with crystal violet 0.1% *(10)*.

Evaluation of effect of neomycin B on RNA synthesis of BRV: In Post-infection treatment assay; at time intervals of zero, 16, 24, 48, and 72h post-infection (PI), the RNA of BRV were extracted from supernatants and monolayers using QIAamp^R viral RNA Mini kit (Qiagen) and RNeasy Plus Mini kit (Qiagen) respectively. The extracted dsRNA of BRV were denatured by heating at 95°C for 5 min and then chilled on ice for 5 min. The cDNA were synthesized and the PCR was performed in a total volume of 50 µl in a sterile 0.2 ml RNase free PCR tube. Amplification reactions were performed on BIO-RAD thermal cycler with the following thermal conditions: initial denaturation at 94°C for 5 min; 40 amplification cycles with denaturation at 94°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 90 s; and a final incubation at 72°C for 10 min. Amplified PCR products were analyzed by electrophoresis on 1.5% ethidium bromide-stained agarose gels at 100 volts for 40 min and viewed under UV illumination *(14)*.

Evaluation of effect of neomycin B on BRV replication using Flow cytometry: The MA-104 cells were harvested, fixed and stained with rotavirus MAb diluted 1/500 in blocking solution (5% non fat milk in PBS). The cellular suspensions were analyzed in Dual laser FACSCalibur flow cytometer *(15)*.

The kinetics of BRV growth in MA-104 treated with different concentration of neomycin after BRV infection: In Post-infection treatment assay; at time intervals of zero, 16, 24, 48, and 72h pi, both supernatants and monolayers were harvested submitted to three cycles of freezing/thawing. The viral titers were determined using plaque assay *(12)*.

Data analysis: Analysis of variance (ANOVA) ($p < 0.05$) was done with PROC GLM of SAS (SAS Institute, Inc.).

RESULTS

Preliminary assessment of Neomycin B cellular cytotoxicity: Neomycin maintained in MA-104 cell cultures for 4 days, at the concentrations of 0.6 to 10 mM did not demonstrate any change in the cells that could be considered toxic effect. However, from 20mM to 100 mM cell rounding and clumping were observed with increasing intensity at higher concentrations of the neomycin (**Figure 1**). Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. The CC_{50} value which is the reduction in cell viability by 50% was 30mM neomycin concentration.

Evaluation of effect of neomycin B on BRV CPE: In Pre-infection treatment experiment, and when virus suspension was pretreated with different concentration of neomycin, no change was observed either in the intensity or progression of CPE in comparison to infected and non-treated cultures. In simultaneous treatment assay and post-infection assay, neomycin had effect on the intensity or progression of CPE with dose dependent manner i.e. low concentration of neomycin had no effect on the rate of CPE of BRV (0.6 and 3 mM), in contrast high concentrations of neomycin 5, 7.5 and 10 mM were able to inhibit the CPE of BRV by 25%, 58.33% and 91.67% respectively (**Table 1**).

Evaluation of effect of neomycin B on BRV replication using IF: Neomycin concentrations at 5, 7.5 and 10 mM were able to reduce the intracytoplasmic fluorescence induced by BRV by 16.67%, 41.67% and 83.33% respectively (**Table 1 and Figure2**).

Evaluation of effect of neomycin B on BRV replication using plaque assay: Low concentration of neomycin (0.6 and 3mM) didn't reduce the number of BRV plaques; in contrast high concentrations of neomycin (5, 7.5 and 10 mM) were able to reduce the number of plaques (5 and 7.5mM) or

completely inhibit the plaques formation (10mM) (**Figure 3**).

Effect of neomycin on RNA synthesis of BRV: At time interval 48 h pi, there was decrease in viral RNA by 209.11 and 241.14 ng/µl in supernatant in virus infected and treated cells with 7.5 and 10mM neomycin respectively. While there was decrease by 157.42 and 217.1 ng/µl in cell-associated in virus infected and treated cells with 7.5 and 10mM neomycin respectively. At the concentrations 5, 7.5 and 10mM neomycin, there was marked decrease in viral RNA by 707.74, 852.67 and 978.62 ng/µl in supernatant at 72h post infection **(Figure 4).**

Evaluation of effect of neomycin B on BRV replication using Flow cytometry: In simultaneous treatment assay, neomycin concentrations of 5, 7.5 and 10 mM were able to reduce the percentage of cell infectivity with BRV by 17.98%, 50.57% and 74.11% respectively. In post-infection treatment assay, neomycin concentrations of 5, 7.5 and 10 mM were able to reduce the percentage of cell infectivity with BRV by 16.21%, 56.72% and 73.94% respectively, **(Table 2 and Figure 5)**.

The kinetics of BRV growth in MA-104 treated with different concentration of neomycin after BRV infection: There was significant decrease in virus titer by 2 logs in cell-associated at 48 h in virus infected and treated cells with 7.5mM neomycin. At the concentrations 7.5 and 10mM neomycin, there was marked decrease in virus titer in supernatant at time interval, 48 and 72h post infection, **(Figure 6)**.

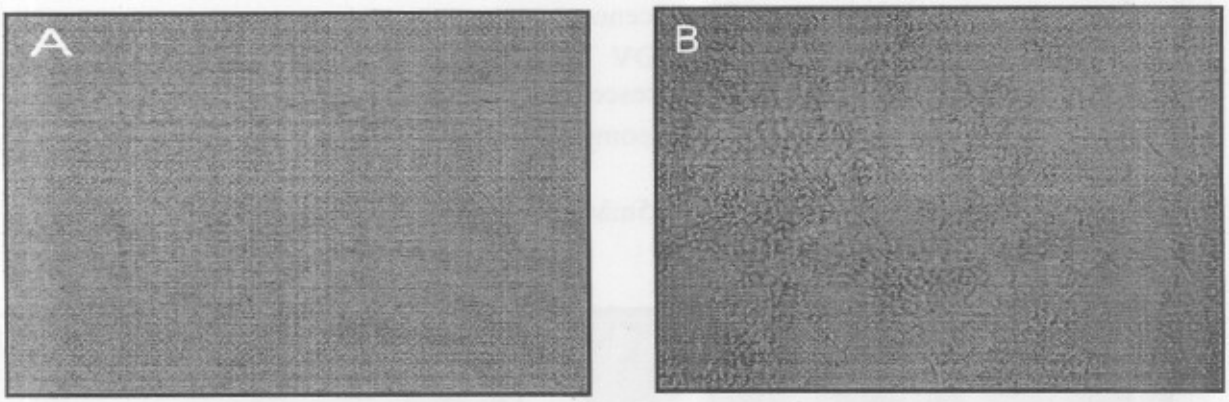

Figure 1. Evaluation of neomycin B cytotoxicity on MA-104.

A- Normal confluent MA-104 cells.

B- MA-104 cells treated with neomycin B showing toxic abnormalities by neomycin B. X 100.

Table 1. Effect of neomycin B on BRV CPE and IF

Neomycin conc. mM	% of CPE	% of reduction in CPE	% of fluorescence of BRV	% of reduction in fluorescence
o.6	100 ± 0 a	0	100 ± 0 a	0
3	91.67 ± 8.3 a	8.33%	100 ± 0 a	0
5	75 ± 14.4 a	25%	83.33 ± 8.3 a	16.67%
7.5	41.67 ± 8.3 b	58.33%	58.33 ± 8.3 b	41.67%
10	8.33 ± 8.3 c	91.67%	16.67 ± 8.3 c	83.33%

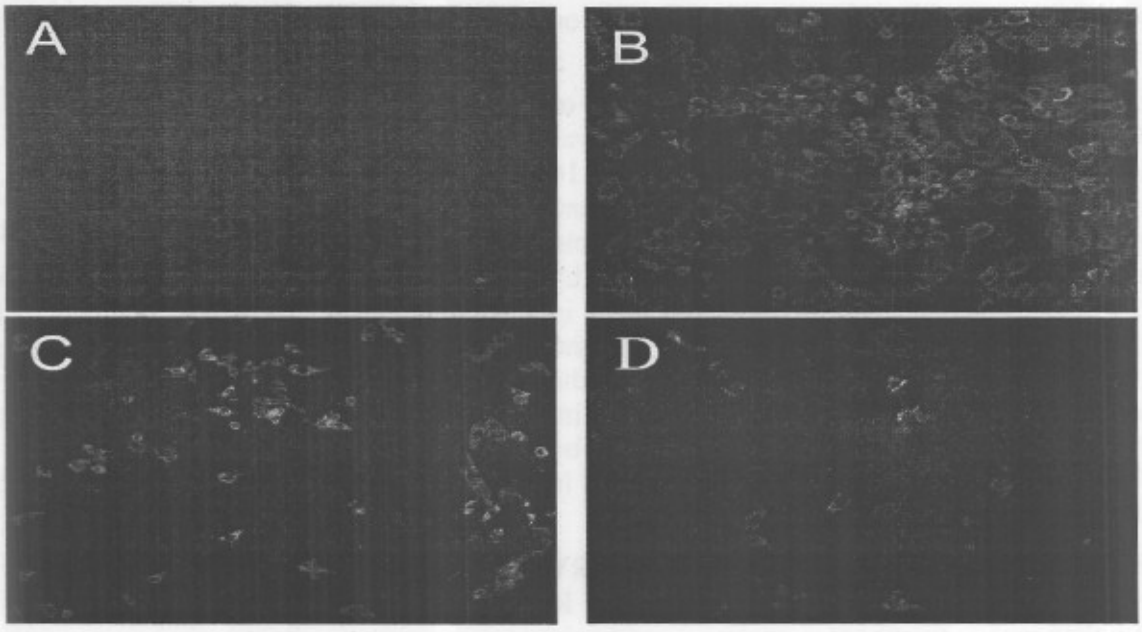

Figure 2. Effect of neomycin on BRV IF.

A- Mock infected cell showing no fluorescence.

B-Virus infected cell stained with NCDV MAb against VP6 labeled with FITC, showing intracytoplasmic greenish yellow fluorescence.

C-Virus infected treated cell with 5mM neomycin showing few intracytoplasmic fluorescence in some cells.

D-Virus infected treated cell with 7.5mM neomycin showing marked decrease in the intracytoplasmic fluorescence. X 100

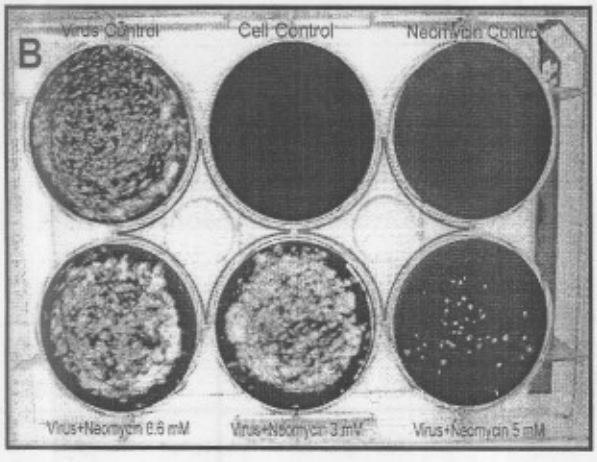

Figure 3. Effect of neomycin B on rotavirus replications: there is difference in number of plaques with dose-dependent decrease.

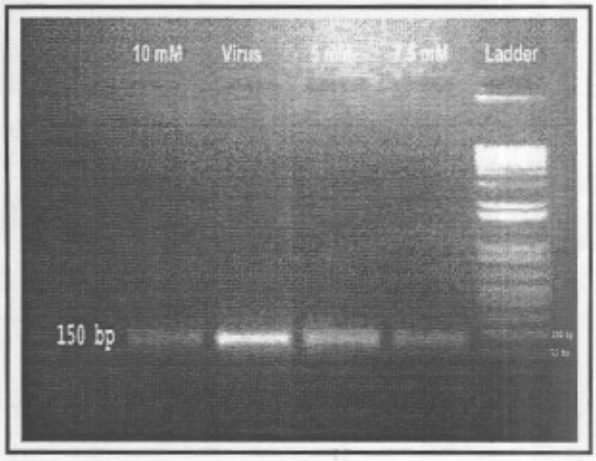

Figure 4. Effect of neomycin B on RNA synthesis of BRV.

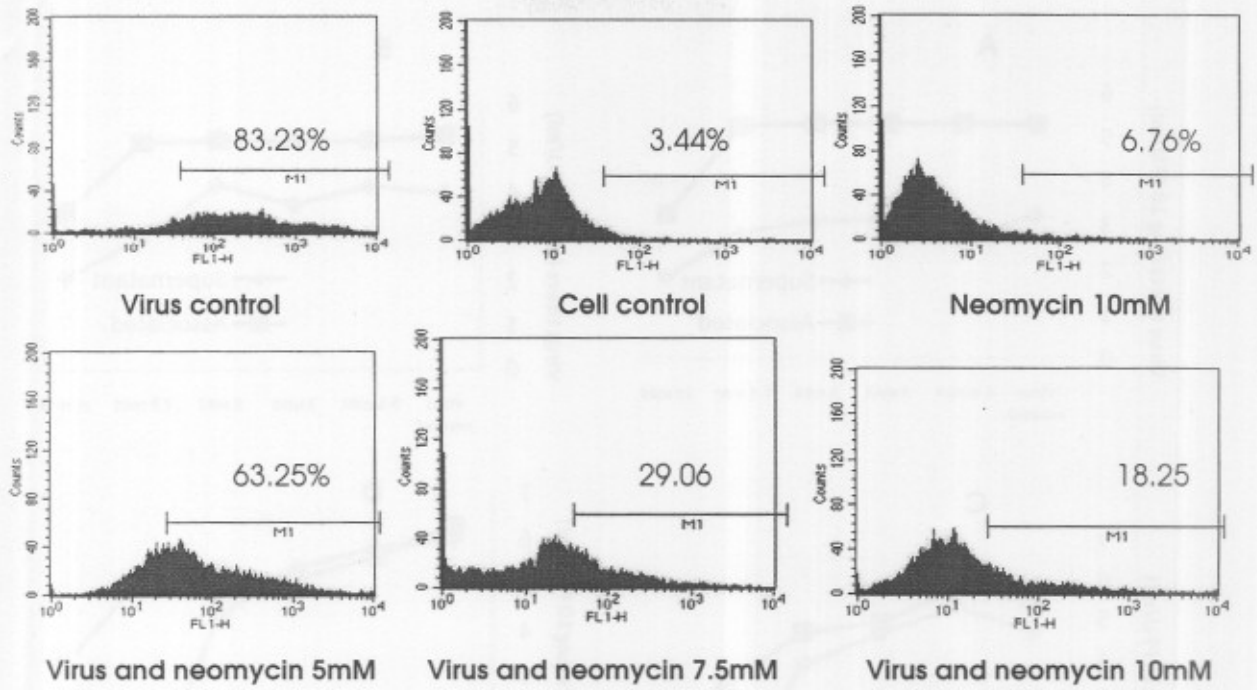

Figure 5. Effect of neomycin B on percentage of BRV infectivity using flow cytometry.

Table 2. kinetic Effect of neomycin on percentage of cell infectivity with BRV by Flow cytometry. The Table represents the mean value of the % of cell infectivity with BRV ± standard error from three independent experiments.

Time post-infection (Hour)	0.6mM	3mM	5mM	7.5mM	10mM	Virus Control	Cell Control
0	1.68 ± 0.25 a	1.68 ± 0.32a	1.84 ± 0.35 a	2.02 ± 0.41 a	1.78 ± 0.29a	2.07 ± 0.31 a	1.56 ± 0.27 a
16	47.35 ± 1.9 a	42.19 ± 3ab	41.49 ± 2.8ab	40.15 ± 2.2ab	35.93 ± 1.3 b	53.50 ± 4.1 a	3.13 ± 0.14 c
24	51.29 ± 4.8 a	48.86 ± 3.2a	41.80 ± 2.8b	33.5 ± 5.6bc	28.10 ± 2.3 c	56.70 ± 2.7 a	3.00 ± 0.45 d
48	35.83 ± 3.3 a	34.81 ± 3.1a	32.58 ± 2.3 a	22.19 ± 2.2b	15.99 ± 1.8b	35.96 ± 4.4 a	3.80 ± 0.51 c
72	78.74 ± 8.43a	77.3 ± 0.96a	65.25 ± 2 ab	33.7 ± 4.64b	20.29 ± 2.0b	77.87 ± 5.36a	4.70 ± 0.89 c

Different subscripts in the same column mean significant differences $p < 0.05$.

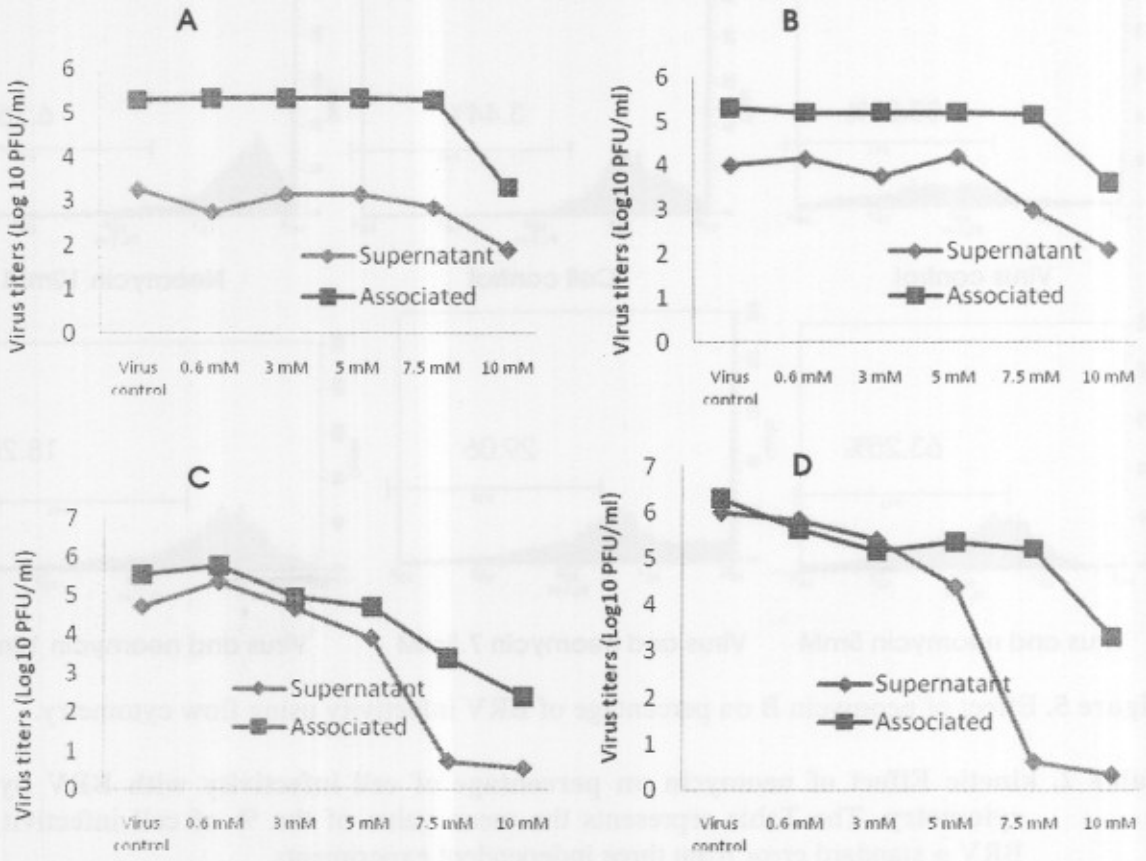

Figure 6. Kinetics of BRV growth in MA-104 treated with different concentration of neomycin after BRV infection. **A**- At 16 h PI. **B**- At 24 h PI. C- At 48 h PI. **D-** At 72 h PI.

DISCUSSION

Rotavirus causes world-wide acute viral gastroenteritis in young animals and children *(16)*. It also results in important economical losses in agriculture due to diarrhea in pigs, sheep and poultry rearing. Newborn calves are susceptible to rotavirus infection during the first weeks of life, thus making it difficult to actively immunize the animals before exposure to the virulent pathogen *(17)*. Our goal was a trial to reduce the risk caused by rotavirus via using of neomycin as chemotherapeutic agent. In Simultaneous treatment assay, It was observed that neomycin B able to inhibit Rotavirus replication when is added to infected cell cultures and kept along the experiment which could be due to an inhibition of viral attachment or penetration as is observed in the case of herpes simplex virus type I *(8)* or inhibition of viral nucleic acid synthesis.

Neomycin had to be present at the time of infection to exert maximal effect, whereas the addition of the antibiotic 1 h post-infection had no effect *(8)*. As results indicated that the neomycin affects at early stages of HSV-1. Thus, when the first steps of infection have occurred, the presence of neomycin does not interfere with the further, normal course of infection.

To determine whether the inhibitory effect of neomycin on rotavirus replication was due to inhibition of the initiation, replication phases of rotavirus replication, the cells were treated with different concentrations of neomycin B varying from 0.6 to 10 mM before and after viral infection.

In *in-vitro* assays when neomycin was added to the cell cultures before, during, after infection and treatment of virus with neomycin (0.6, 3, 5, 7.5 and 10mM) results indicated that pre-incubation of virus with neomycin for 30 minutes at 37°C and pretreatment of MA-104 with neomycin does not affect replication of BRV. The inhibition of BRV replication occurs when the drug is added to the MA-104 after infection indicates that the drug affects a step of viral replication other than penetration suggesting that the effect could involve a step related to the synthesis of the viral genome which evaluated by IF, plaque assay and flow cytometry. The drugs are simultaneously interacting with the lower and upper stem regions of HIV *(9)*. Rotavirus RNA synthesis of both plus and minus RNA involves the recognition of RNA promoters. In the particular case of minus-strand synthesis, according to the predicted structure at the 3 end of the viral RNA seems to suggest the presence of a stem and loop structure similar to the aptamers, which have been shown to be able to bind either neomycin B or tobramycin *(18)*.

RNA can be considered as a target for therapy and the detailed understanding of the mode of action of RNA-binding drugs should aid the rational design of novel therapeutics *(19)*. To test the hypothesis that neomycin inhibition of viral replication occurs through inhibition of viral RNA production, the effect of neomycin on RNA levels of infected cells with BRV was estimated. By treatment of cells with neomycin using concentrations of 5, 7.5 and 10mM neomycin there was marked decrease in viral RNA by 707.74, 852.67 and 978.62 ng/µl in supernatant at 72h post infection respectively. These results explain the hypothesis that viral RNA considered a target for Neomycin. These results are consistent with previous study which showed that the neomycin has a cleavage effect on HIV RNA oligonucleotides *(20)*.

In kinetics of BRV growth in MA-104 treated with different concentration of neomycin after BRV infection, there was significant decrease in virus titer by 2 logs in cell-associated at 48 h in virus infected and treated cells with 7.5mM neomycin. At the concentrations 7.5 and 10mM neomycin, there was marked decrease in virus titer in supernatant at time interval, 48 and 72h post infection **(Figure 6).**

Our result indicated that 10mM neomycin concentration (9mg neomycin in 1 ml media) was within the range of therapeutic dose of neomycin and reduced BRV replication by 73.94%. The therapeutic dosages of neomycin are 10 to 20 mg/kg body weight for cattle, 10 mg/ kg body weight for sheep and 10 to 30 mg/kg body weight for chickens, turkeys and ducks. The duration of treatment is 3 to 7 days for poultry and up to 14 days for large animals *(21)*.

In conclusion, it was observed that neomycin B is an inhibitor of BRV replication *in-vitro* particularly when the drug is added to infected cells and kept along the experiment; pre-incubation of BRV with neomycin and pretreatment of MA-104 with neomycin do not affect replication of BRV. It was detected that neomycin has inhibitory effect on rotavirus replication *in-vitro* with dose-dependent manner; as concentration of neomycin (0.6, and 3mM) didn't affect BRV replication, while concentration (5, 7.5 and 10mM) has been shown to reduce the rate of BRV replication.

Further studies on the mechanism of neomycin inhibition to rotavirus replication, on the effect of neomycin on BRV at different time in-vivo (lab animal and natural host), human rotavirus *in-vitro*, on rotavirus protein expression, and on live attenuated strain of BRV need to be done.

REFERENCES

1. ***Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, Mcgeoch DJ, Pringle CR and Wickner RB (ed.) (2000):*** Virus taxonomy classification and nomenculture of viruses. Seventh report of the international committee on taxonomy of viruses. Academic press. San. Diego, Calif.

2. ***Fagiolo A, Cristina R, Ogla L, and Antonio B (2005):*** In: Antonio, B. (Ed.), Buffalo Pathologies. Buffalo Production and Research, FAO, Rome, pp. 249–296.

3. ***USDA (2006):*** Characterization and enhancement of immune responses of calves. Annual Report FY 2006. United States Department of Agriculture, Agricultural Research Services, National Animal Disease Center, Ames, IA, pp. 1-5.

4. ***Hassan AA, El-Bakry M, Salama AA, Zeidan SM and Galal SM (2007):*** Isolation, identification and classification of some viruses induced neonatal calf diarrhea. 5th Int. Sci. Conf., Mansoura, 10-11 April: 1463-1479.

5. ***Schroeder R, Waldsich C and Wank H (2000):*** Modulation of RNA function by aminoglycoside antibiotics. EMBO J. 19: 1-9.

6. ***Davies J, Gorini L and Davis BD (1965):*** Misreading of RNA code words induced by aminoglycoside antibiotics. Mol. Pharmacol. 1: 93-106.

7. ***Herold BC and Spear PG (1994):*** Neomycin inhibits glycoprotein C (gC)-dependent binding of herpes simplex virus type 1 to cells and also inhibits postbinding events in entry. Virology 203: 166–171

8. ***Langeland N, Holmsen H, Lillehaug JR and Haarr L (1987):*** Evidence that neomycin inhibits binding of Herpes simplex virus type 1 to the cellular receptor. J Virol 61: 3388–3393

9. ***Wang S, Huber PW, Cui M, Czarnik AW and Mei HY (1998):*** Binding of neomycin to the TAR elements of HIV-1 RNA induces dissociation of Tat protein by an allosteric mechanism. Biochemistry 37: 5549–5557

10. ***Hasenack BS, Valéria M, Botelho J, Lauretti FL, de Melo F, Orlandi JM, Linhares REC, Ueda M and Nozawa CM (2002):*** The Effect of Concanavalin A on the Replication of Rotavirus (SA-11) in Cell Culture. Brazilian Archives of Biology and Technology. 45 (2): pp. 125-135.

11. ***Fotakis G and Timbrell JA (2006):*** In vitro cytotoxicity assays: Comparison of LDH, neutral red, MTT and protein assay in hepatoma cell lines following exposure to cadmium chloride. Toxicology Letters. 160: 171–177

12. ***Carriel-Gomes MC, Kratz JM, Müller VDM, Barardi CRM and Simões CMO (2006):*** Evaluation of antiviral activity in hemolymph from oysters Crassostrea rhizophorae and Crassostrea gigas. Aquat. Living Resour. 19: 189–193.

13. ***Bosch A, Lucena F, Diez JM, Gajardo R, Blasi M and Jofre J (1991):*** Human enteric viruses and indicator microorganisms in a water supply associated with an outbreak of infectious hepatitis. J. Am. Water Works Assoc. **83:** 80–83.

14. ***Logan C, O'Leary JJ and O'Sullivan N (2006):*** Real-Time Reverse Transcription-PCR for Detection of Rotavirus and Adenovirus as Causative Agents of Acute Viral Gastroenteritis in Children. J. Clin. Microbiol. 44 (9): 3189–3195.

15. ***Abad FX, Pinto´ RM and Bosch A (1998):*** Flow Cytometry Detection of Infectious Rotaviruses in Environmental and Clinical Samples. Applied and Environ. Microbiol. 64(7): 2392–2396.

16. ***Adah MI, Nagashima S, Wakuda M and Taniguchi K (2003):*** Close Relationship between G8-Serotype Bovine and Human Rotaviruses Isolated in Nigeria. J. Clin. Microbiol. 41 (8):3945–3950

17. ***Kim Y, Nielsen PR, Hodgins D, Chang KO and Saif LJ (2002):*** Lactogenic antibody responses in cows vaccinated with recombinant bovine rotavirus-like particles (VLPs) of two serotypes or inactivated bovine rotavirus vaccines. Vaccine, 20: 1248-1258

18.Cho J and Rando RR (1999): Specificity in the binding of aminoglycosides to HIV-RRE RNA. Biochemistry 38: 8548–8554

19.Park WKC, Auer M, Jaksche H and Wong CH (1996): Rapid combinatorial synthesis of aminoglycoside antibiotic mimetics: use of a polyethylene glycol-linked amine and a neamine-derived aldehyde in multiple component condensation as a strategy for the discovery of new inhibitors of the HIV RNA rev responsive element. J. Am. Chem. Soc., 118, 10150–10155.

20.Belousoff MJ, Graham B, Spiccia L and Yitzhak T (2009): Cleavage of RNA oligonucleotides by aminoglycosides. *Org. Biomol. Chem.* 7: 30–33

21.EMEA (2002): Committee for Veterinary Medicinal Products: Neomycin: Summary Report. The European Agency for the evaluation of medicinal products, Veterinary medicine and Inspections.

الملخص العربي

دراسات فيرولوجية وجزيئية على فيروس الروتا

احمد عبدالسميع حسن علي، على عبد الرشيد على سلامة،
سيد محمد زيدان،* شيماء محمد جلال محمد منصور
قسم الفيرولوجيا- كلية الطب البيطرى- جامعة الزقازيق- الزقازيق- مصـــــر
*** قسم الأمراض المشابهة للطاعون البقري معهد بحوث الأمصال واللقاحات البيطرية العباسية – القاهرة**

يعتبر فيروس الروتا البقرى من أهم العوامل المسببة لالتهاب المعدة والأمعاء الحاد فى العجول فى جميع أنحاء العالم. النيوميسين-ب مضاد حيوي من مجموعة الأمينوجليكوسيد وقد اثبتت الدراسات السابقة أنه مثبط لتكاثر فيروسى الايدز والعقبول . ولقد أجريت هذه الدراسة الحالية لتقييم تأثير النيوميسين-ب على فيروس الروتا على خلايا MA-104 بواسطة اختبار الفلوروسنت المناعى و اختبار البلاك والفلوسيتومترى. يؤثر النيوميسين على نسبة التأثير الضار لفيروس الروتا على الخلايا. وجد أن تركيزات النيوميسين ٥ و ٧,٥ و ١٠ ملي مول كانت لها القدرة على تقليل نسبة الوميض الفلوروسنتى داخل السيتوبلازم بنسبة ١٦,٦٧ ٪ ، ٤١,٦٧٪ و ٨٣,٣٣ ٪ على التوالي. يؤثر النيوميسين على الحمض النووي تأثيرا ملحوظا. وجد أيضا أن تركيزات النيوميسين ٥ و ٧,٥ و ١٠ ملي مول كانت لها القدرة على تقليل نسبة اصابة الخلية بفيروس الروتا بنسبة ١٦,٢١ ٪ ، ٥٦,٧٢ ٪ و ٧٣,٩٤ ٪ على التوالي. وتم قياس عيارية الفيروس باختبار البلاك ، وقد كان هناك انخفاضا ملحوظا في عيارية الفيروس وذلك عند ٤٨ و ٧٢ ساعة بعد العدوى مع التركيزات ٧,٥ و١٠ ملي مول من النيوميسين . مما سبق اتضح أن النيومايسين بى مثبط لتكاثر فيروس الروتا فى المعمل عندما يضاف خلال دورة كاملة من العدوى الفيروسة ؛ معالجة الفيروس فى الحضانة بالنيوميسين ، والمعالجة المسبقة للخلايا بالنيوميسين لا يؤثرا على تكاثر فيروس الروتا.