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Effect of some inhibitors on neuraminidase of Newcastle disease virus Kudu 113 strain

Sunday Blessing Oladele[1]*, Paul Abdu[2], Andrew Jonathan Nok[3], King Akpofure Nelson Esievo[1], and Nicodemus Maashin Useh[1]

[1]*Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

[3]*Department of Biochemistry, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria*

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ABSTRACT

A total of two hundred samples from dialysed neuraminidase of Newcastle disease virus (NDV) Kudu 113 strain were used for the experiment. Fifty samples each were used for various concentrations of paranitrophenyl oxamic acid (PNPO), salicyl oxamic acid (SOA), silver nitrate ($AgNO_3$) and ethylene diamine tetra acetic acid (EDTA), to test their inhibition effects on the neuraminidase activity of NDV Kudu 113 strain *in vitro*, using thiobarbituric acid assay method. Of these inhibitors, $AgNO_3$ had the highest inhibition effect on the neuraminidase activity of NDV Kudu 113 strain. Silver nitrate reduced the activity of this enzyme from 140.24 µMol/min for enzyme without $AgNO_3$ to 32.36 µMol/min when $AgNO_3$ was added to the enzyme at a concentration of 0.47 M. Paranitrophenyl oxamic acid had the least inhibition effect on the neuraminidase activity of NDV Kudu 113 strain at a concentration of 0.12 M. This inhibitor reduced the activity of the enzyme from 97.08 µMol/min for enzyme without PNPO to 91.70 µMol/min when PNPO was added to the enzyme at a concentration of 0.12 M. Ethylene diamine tetra acetic acid and SOA also greatly inhibited the activity of this enzyme. Salicyl oxamic acid reduced the activity of NDV Kudu 113 strain neuraminidase from 140.24 µMol/min for enzyme without SOA to 59.33 µMol/min when SOA was added at a concentration of 0.47 M. Similarly, EDTA reduced the activity of this enzyme from 124.06 µMol/min for

* Contact address:
Dr. Sunday Blessing Oladele, Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria; Phone: Zaria-069-551358; E-mail:blessola@abu.edu.ng

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enzyme without EDTA to 75.51 µMol/min when EDTA was added to the enzyme at a concentration of 0.35 M. In all the inhibitors examined, it was observed that their effects on the activity of NDV Kudu 113 strain neuraminidase increased as the concentrations of the inhibitors increased. It was concluded that PNPO, SOA, EDTA and $AgNO_3$ inhibited the neuraminidase activity of NDV Kudu 113 strain *in* vitro. Therefore, further studies are needed to see if these inhibitors could be of use, after proper clinical trials, as potential agents for reducing Newcastle disease (ND) infections in poultry.

Key words: inhibitor, neuraminidase, Newcastle disease virus

Introduction

Since the first documented outbreak of ND in Nigeria by HILL et al. (1953), the disease has remained a notable poultry problem in the country. The present interest in the disease which appears to be an old problem, arose up as a result of increasing private and public investment in the poultry industry in Nigeria, hence the need to reduce ND infections in poultry.

Both virulent and avirulent strains of NDV have been isolated from wild and domesticated birds, and some of these birds have been incriminated as sources of infection to intensively reared poultry (ALEXANDER et al., 1984; EZEIFEKA et al., 1992; ROY et al., 2000).

Newcastle disease virus Kudu 113 strain was isolated from ducks in Nigeria. Based on pathogenicity indices, clinical and post-mortem findings in chickens, this NDV was classified as a velogenic strain (ECHEONWU et al., 1993). It is thought that this NDV strain is one of the virulent strains, causing infections in our chickens. It is therefore important to study the effect of inhibitors on neuraminidase, an enzyme believed to play an important role in infection of many diseases (CORFIELD, 1992).

It is known that neuraminidases facilitate the production of infectious particles *in vitro*, by removing sialic acid residues, and exposing an appropriate cleavage site in cell culture (SCHULMAN and PALESE, 1977). Studies also showed the importance of functional influenza neuraminidase in repeated cycles of infection when the enzyme was inhibited by a potent chemical inhibitor, thus resulting in the inhibition of plaque formation of influenza virus that was cultured on bovine kidney cells (MEINDL et al., 1974; PALESE and COMPANS, 1976).

The use of specific neuraminidase inhibitors is also of importance when assaying for unknown neuraminidase activity. This is because they can be

used to discriminate between mixtures of neuraminidases (CORFIELD et al., 1981).

Since neuraminidases are of great biological and pathological significance (HEUERMANN et al., 1991), application of inhibitors for the treatment of diseases in which neuraminidases are involved in pathogenesis of infection will be of great importance.

Although NDV Kudu 113 strain has been classified as a velogenic strain (ECHEONWU et al., 1993), this is the first report on the effects of inhibitors on neuraminidase activity of this strain of NDV. Therefore, the objective of this study was to examine the effects of some inhibitors on NDV Kudu 113 strain neuraminidase *in vitro*.

Materials and methods

Virus strain. For this experiment, Newcastle disease virus Kudu 113 strain stock was obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria. The virus was isolated from ducks in Nigeria, and the pathogenicity indices have been determined (ECHEONWU et al., 1993).

Virus growth. One vial of NDV Kudu 113 strain stock was diluted with 2.0 ml of sterile cold phosphate buffer saline, pH 7.2. Procaine penicillin at 200 I.U./ml and streptomycin at 200 mg/ml were added to the final concentrations according to the methods of ALLAN et al. (1978). About 0.2 ml of the solution was inoculated into the allantoic cavity of 20 ten-day-old embryonated hen's eggs. After inoculation the eggs were incubated in an electric egg turner incubator (OVO-LUX, BP 500 4000 Liege Belgium) at 37.5 °C for 6 days. The eggs were candled twice daily to check for dead or dying embryos.

Virus isolation. Dead embryos were chilled to 4 °C before the allantoic fluids were harvested at 72 hours post-inoculation into embryonated hen's eggs. Presence of the virus in the allantoic fluids was detected by haemagglutination test (ALLAN et al., 1978). Thereafter, the allantoic fluids were clarified by low speed centrifugation at 1000 g for 5 minutes, after which, the sediment was stored at –20 °C until used.

Ammonium sulphate fractionation of crude neuraminidase of Newcastle disease virus Kudu 113 strain. Ammonium sulphate [$(NH_4)_2SO_4$] fractionation of crude NDV Kudu 113 strain was carried out to determine the fraction with the highest activity, after modifying the procedures of NEES et al. (1975), as described below: The 10%, 20%, 30%, 40%, 50%, 55%, 60%, 70% and 80% $(NH_4)_2SO_4$ precipitation of the crude enzyme was carried out by adding 1 ml each of the crude allantoic fluid containing the virus to 56 mg, 70 mg, 176 mg, 242 mg, 313 mg, 351 mg, 390 mg, 472 mg and 820 mg of $(NH_4)_2SO_4$, respectively in 10 ml capacity polythene tubes. All the $(NH_4)_2SO_4$ fractions were set in duplicates. The mixture for each fraction was then homogenized by violent agitation to apparent homogeneity and centrifuged using refrigerated centrifuge (MSE, mistral 4L) at 9,000 g for 1 hour at 4 °C. The supernatants were aspirated using Pasteur pipette, after which 0.5 ml of sodium phosphate citrate buffer, pH 5.0 was added to each sediment.

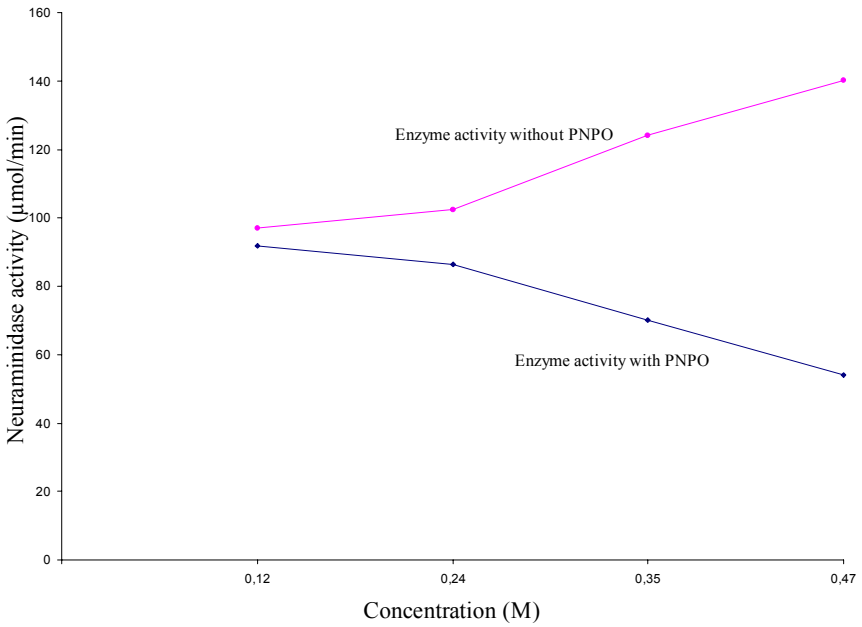

Fig. 1. Effect of paranitrophenyl oxamic acid (PNPO) on Newcastle disease virus Kudu 113 strain neuraminidase

Table 1. Specification of myomorphus mammals examined by renoculture and microscopic agglutination acording to the trapping area with corresponding results

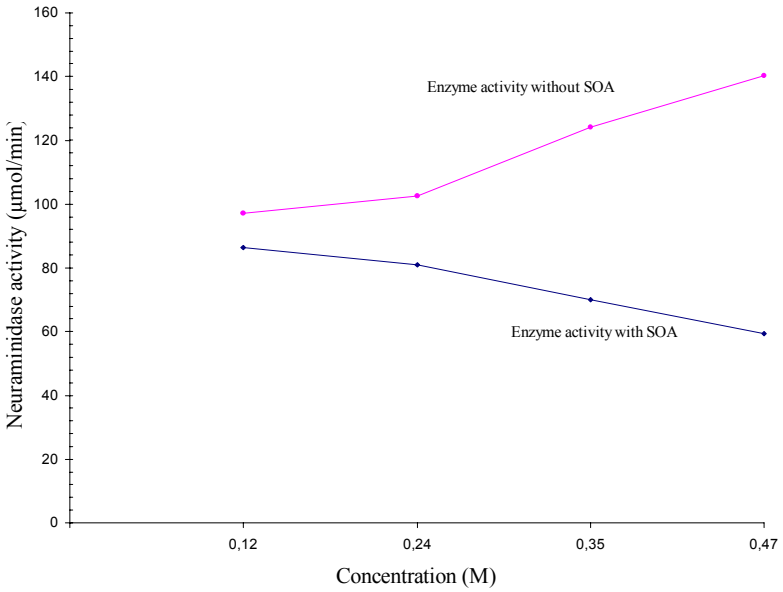

Fig. 2. Effect of salicyl oxamic acid (SOA) on Newcastle disease virus Kudu 113 strain neuraminidase

Neuraminidase activity was assayed in the various fractions of $(NH_4)_2SO_4$ fractionation after modifying the method of AMINOFF (1961), as follows: 0.25 ml of sodium periodate was added to each sediment and the mixture was shaken and incubated for 20 minutes in a water bath at 37 °C, after wich, 0.1 ml of sodium arsenite was added. A brown color immediately appeared. After which 1.0 ml of thiorbarbituric acid was added. The mixture was shaken and put into boiling water for 10 minutes. A pink colour appeared after 10 minutes. Thereafter, the test tubes containing all the mixture were cooled by placing them under a running tap. Following this 2.5 ml of acid-butanol was added and vigorously shaken. The mixture was centrifuged at 1000 g for 5 minutes. The supernatant for each mixture was carefully aspirated into cuvettes using Pasteur pipettes. Absorbance was read against blank on Sp6-400 spectrophotometer at 549 nm.

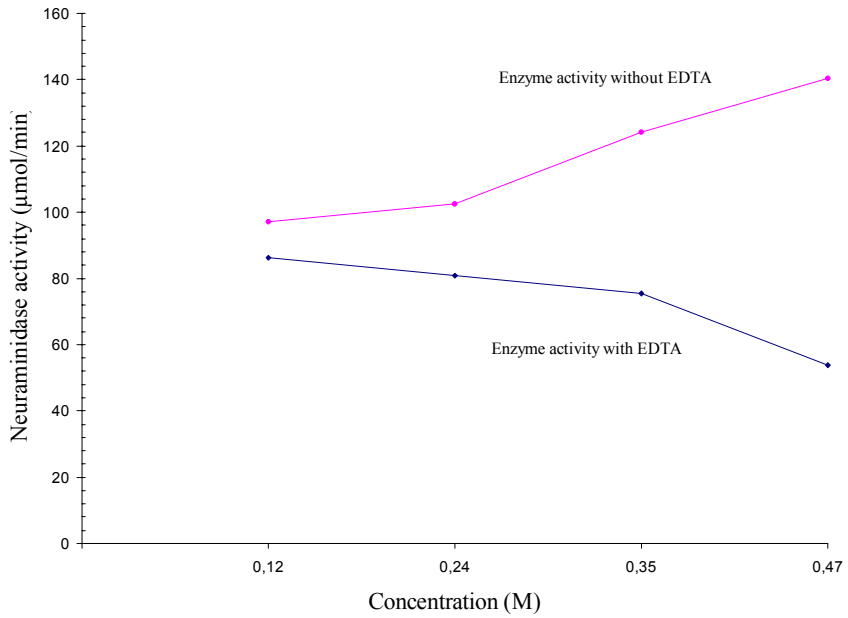

Fig. 3. Effect of ethelene diamine tetra acetic acid (EDTA) on Newcastle disease virus Kudu 113 strain neuraminidase

Dialysis of Newcastle disease virus Kudu 113 strain neuraminidase with the highest activity. The enzyme fraction with the highest activity was dialysed after modifying the method of HEUERMANN et al. (1991). About 30 ml of $(NH_4)_2SO_4$ fraction with the highest enzyme activity was transferred into a 20 cm long and 6 cm wide dialysis bag and immersed in a 2-litre capacity dialysis beaker containing 2 litres of sodium phosphate citrate buffer, pH 5.0. The dialysis beaker and its contents were placed on a speed plate magnetic stirrer at 4 °C for 28 hours.

Effects of inhibitors on the activity of dialysed neuraminidase of Newcastle disease virus Kudu 113 strain. A total of 200 samples of dialysed neuraminidase of NDV Kudu 113 strain were used for the experiment. Fifty samples each were used for four inhibitors, which included

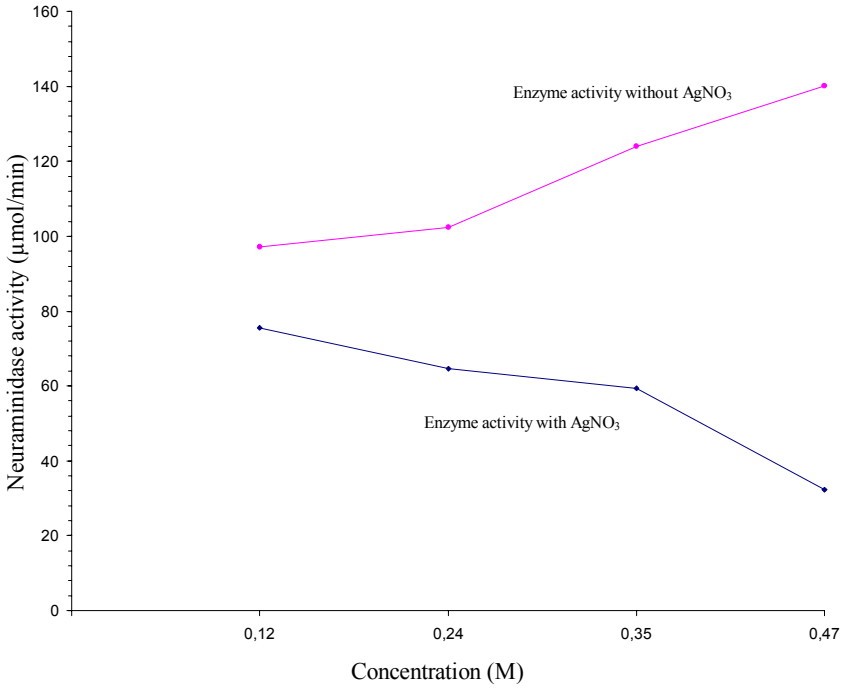

Fig. 4. Effect of silver nitrate (AgNO3) on Newcastle disease virus Kudu 113 strain neuraminidase

PNPO, SOA, $AgNO_3$ and EDTA at concentrations of 0.12 M, 0.24 M, 0.35 M and 0.47 M, for each inhibitor used for the experiment. The effects of inhibitors on this enzyme were carried out by mixing 10 µl each of the dialysed NDV Kudu 113 strain neuraminidase in a set of 200 test tubes with the various concentrations of the above mentioned inhibitors. The enzymatically librated N-acetylneuraminic acid (neuraminidase activity) was determined by using thiobarbituric acid assay method of AMINOFF (1961). Neuraminidase activity was also determined for control samples without inhibitors.

Results and discussion

The results showed that PNPO inhibited the activity of NDV Kudu 113 strain neuraminidase. The rate of inhibition was proportional to the concentration of the inhibitor. The PNPO reduced the activity of the enzyme from 97.08 μMol/min for enzyme without PNPO to a value of 91.70 μMol/min for enzyme incubated with PNPO at a concentration of 0.12 M. Similarly, enzyme activity was reduced from 140.24 μMol/min for enzyme without PNPO to the activity of 53.94 μMol/min at a concentration of 0.47 M for enzyme incubated with PNPO (Fig. 1).

For SOA, the concentration of 0.47 M induced inhibition of the enzyme activity by reducing the activity of NDV Kudu 113 strain neuraminidase from 140.24 μMol/min to 59.33 μMol/min, while the concentration of 0.12 M induced inhibition of the enzyme activity from 97.08 μMol/min to 86.30 μMol/min (Fig. 2).

Ethylene diamine tetra acetic acid also inhibited NDV Kudu 113 strain neuraminidase. The rate of inhibition of EDTA was proportional to the concentrations of the inhibitor. At aconcentration of 0.12 M the activity of the enzyme was 10.78 μMol/min lower than the activity obtained for enzyme without EDTA. Also, at aconcentration of 0.47 M the activity of the enzyme was 86.30 μMol/min lower than the activity of the enzyme obtained for enzyme without EDTA (Fig. 3).

Silver nitrate inhibited the activity of NDV Kudu 113 strain neuraminidase between concentrations of 0.12 M and 0.47 M. The activity of the enzyme with $AgNO_3$ at concentrations of 0.12 M and 0.47 M were 75.51 μMol/min and 32.36 μMol/min, respectively. These values were lower than the corresponding activities of 97.08 μMol/min and 140.24 μMol/min for enzyme without $AgNO_3$ (Fig. 4).

The results of this study showed that EDTA inhibited the activity of NDV Kudu 113 strain neuraminidase *in vitro*. This is in accord with the findings of KESSLER et al. (1977), and MULLER and HINZ (1978) on the effects of EDTA on neuraminidases of human parainfluenza 1 virus and *Haemophilus avium*, respectively.

Similarly, in this study, PNPO, SOA and $AgNO_3$ inhibited the activity of NDV Kudu 113 strain neuraminidase. This result agrees with the findings

of USEH (2002) who found appreciable inhibition of neuraminidase activity of *Clostridium chauvoei* (Jakari strain) with these inhibitors. HEUERMANN et al. (1991) also found appreciable inhibition of bacterial neuraminidases with N-(4-nitrophenyl) oxamic acid.

In conclusion, this study has shown that PNPO, SOA, EDTA and $AgNO_3$ inhibited the activity of NDV Kudu 113 strain neuraminidase *in vitro*. Further studies are therefore needed to see if these inhibitors could be of use, after proper clinical trials, as potential agents for reducing ND infections in poultry.

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SADR•AJ

Iz soja Kudu 113 virusa newcastleske bolesti pripremljeno je 200 uzoraka dijalizirane neuraminidaze u svrhu istraživanja inhibicijskog učinka različitih koncentracija para-nitrofeniloksaminske kiseline, saliciloksaminske kiseline, srebrnog nitrata i etilendiamin-tetraoctene kiseline. Učinak svake navedene tvari istražen je na 50 uzoraka neuraminidaze postupkom koji se izvodi pomoću tiobarbiturne kiseline. Najveći inhibicijski učinak na aktivnost neuraminidaze imao je srebrni nitrat. On je u 0,47 M koncentraciji smanjio učinak enzima sa 140,24 µM/min na 32,36 µM/min. Para-nitrofeniloksaminska kiselina imala je najslabiji inhibicijski učinak na neuraminidaznu aktivnost pretraživanog soja. Ona je u 0,12 M koncentraciji smanjila aktivnost neuraminidaze s 97,08 µM/min na 91,70 µM/min. Za etilendiamintetraoctenu kiselinu i saliciloksaminsku kiselinu utvrđena je visoka inhibicijska sposobnost. Saliciloksaminska kiselina u 0,47 M koncentraciji smanjila je aktivnost neuraminidaze sa 140,24 µM/min na 59,33 µM/min. Etilendiamintetraoctena kiselina u 0,35 M koncentraciji smanjila je aktivnost enzima sa 124,06 µM/min na 75,51 µM/min. Inhibicijska aktivnost svih enzima povećavala se s njihovom koncentracijom. Zaključuje se da su sve rabljene tvari *in vitro* zakočile neuraminidaznu aktivnost soja Kudu 113 virusa newcastleske bolesti. Potrebna su daljnja istraživanja za njihovu moguću praktičnu primjenu u sprečavanju newcastleske bolesti.

Ključne riječi: inhibitori, neuraminidaza, virus newcastleske bolesti