



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF NEWCASTLE DISEASE VIRUS STRAINS AF 2240 AND
V4-UPM ON CYTOLYSIS AND APOPTOSIS OF LEUKEMIA CELL LINES**

AIED MOHAMMED AL-ABSI

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By

AIED MOHAMMED AL-ABSI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

September 2008



Specially dedicated to

My beloved parents

My loving wife

My daughters

My brothers

My sisters

My country



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the Degree of Doctor of Philosophy

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Chairman: Professor Abdul Manaf Ali, PhD

Faculty: Biotechnology and Biomolecular Sciences

Newcastle disease virus (NDV) is a member of the *Paramyxoviridae* that has caused severe economic losses in the poultry industry worldwide. Several strains of NDV were reported to induce cytolysis to cancerous cell lines. In this study, two NDV isolates namely, AF 2240 and V4-UPM were evaluated for their anti-leukemic properties against four leukemic cell lines - HL60 (Promyelocytic leukemia), WEHI 3B (Mouse myelomonocytic leukemia), CEMSS (Human T-lymphobalstic leukemia) and K562 (Erythromyelobalstic leukemia). The cytolytic effects of NDV strains AF 2240 and V4-UPM towards WEHI 3B, HL60, CEMSS and K562 cell lines were determined using microtetrazolium (MTT) assay. The cytolytic dose - fifty percent (CD_{50}) for WEHI 3B, HL60 and CEMSS treated with AF 2240 strain were 2, 25, 16 HAU, respectively, while the CD_{50} for WEHI 3B, HL60 and CEMSS treated with V4-UPM were 8, 110 and 64 HAU, respectively.

Comparatively, both NDV strains showed very low cytolytic activity against K562 and non-leukemic cell lines namely, 3T3 (mouse fibroblasts), mouse lymphocytes



and human peripheral lymphocytes. Further studies were done to observe the morphological changes in the WEHI 3B treated cells using light, transmission and scanning electron microscopes. The apoptosis and necrosis were examined under fluorescence microscope, where the cells were stained with acridine orange (AO) and propidium iodide (PI). The treated cells with NDV strains AF 2240 and V4-UPM showed apoptotic features such as cell shrinkage, cell blebbing, and formation of apoptotic bodies compared to the control cells that did not express any features for apoptosis and necrosis. The early apoptosis was also observed under fluorescence microscope, where the cells were stained with Annexin V and PI.

The virus effect on cell proliferation was determined by MTT assay and BrdU techniques. Furthermore, at molecular level, both NDV strains caused internucleosomal DNA cleavage producing a multiple of 180-200 bp fragments, that were visible as a ladder on the agarose gel. Early apoptosis was also observed using Annexin V flow cytometry method. The percentage of apoptosis for WEHI 3B cells treated with NDV strains AF 2240 and V4-UPM had increased with time. Cell cycle and apoptosis were also determined using flow cytometry PI method. Both NDV strains were not able to arrest WEHI 3B at specific cell cycle phases using flow cytometry PI method.

In this study anti-leukemic activity of both NDV strains was evaluated on BALB/c mice induced leukemia with WEHI 3B cells. A day later they were treated with NDV strains AF 2240 and V4-UPM and arabinocytocine, a commercial drug, as positive control. The mice groups treated with arabinocytocine, NDV strains AF 2240 and V4-UPM showed significant killings ($p < 0.05$) of leukemic cells compared to the



mice group without any treatment. The total white blood cell and percentage of blasts cell in the blood, bone marrow and spleen smears were significantly low ($p < 0.05$) in mice treated with the arabinocytocine, NDV strains AF 2240 and V4-UPM compared to the mice group without treatment, that showed high number of leukemia. Spleen and liver weights were significantly low ($p < 0.05$) in the mice groups treated with arabinocytocine, NDV strains AF 2240 and V4-UPM compared to the mice group without treatment, that showed significant ($p < 0.05$) splenomegaly and hepatomegaly.

Histopathological studies carried out had confirmed haematological results. From the results obtained, the mice groups treated with both NDV stains AF 2240 and V4-UPM showed similar results as arabinocytocine, which is a commercial drug for leukemia. Immunoperoxidase staining, haemagglutination test and real time PCR were carried out to detect NDV in mice organs after treatment with NDV. The results showed no NDV particles were detected in the organs. This study showed that NDV strains AF 2240 and V4-UPM had caused cytolytic effects against WEHI 3B leukemia cell line *in vitro* and *in vivo*.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah.

**KESAN VIRUS PENYAKIT NEWCASTLE STRAIN AF 2240 DAN V4-UPM
TERHADAP SITOTOSIK DAN APOPTOSIS PADA SEL LEUKEMIA**

Oleh

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September 2008

Pengerusi: Profesor Abdul Manaf Ali, PhD

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Virus penyakit Newcastle (NDV) merupakan ahli *Paramyxoviridae* yang menyebabkan banyak kes kemelesetan ekonomi di industri penternakan di seluruh dunia. Beberapa strain NDV telah dilaporkan dapat mencetuskan sitolisis terhadap sel barah. Dalam pengajian ini, dua strain NDV yang diasingkan adalah AF2240 dan V4-UPM telah diujikan kesan anti-leukemic terhadap empat jenis sel leukemia yang dinamakan HL60 (Promyelocytic leukaemia), WEHI 3B (myelomonocytic leukaemia tikus), CEMSS (T-lymphoblastic leukaemia manusia) dan K562 (Erythromyeloblastic leukaemia). Kesan sitotosik oleh NDV strain AF2240 dan V4-UPM terhadap sel WEHI 3B, HL60, CEMSS dan K562 telah ditentukan melalui analisa methyl thiazolyl tetrazolium (MTT). Kesan sitotosik lima puluh peratus (CD_{50}) untuk WEHI 3B, HL60 dan CEMSS yang dirawat dengan AF2240 adalah 2, 16, 35 HA unit masing-masing, manakala CD_{50} untuk sel WEHI 3B, HL60 dan CEMSS yang dirawat dengan V4-UPM adalah 8, 32 dan 64 HA unit masing-masing.



Secara perbandingan, kedua-dua strain NDV menunjukkan kesan sitotoksik yang sangat rendah terhadap K562 dan sel bukan leukemia yang dinamakan, 3T3 (fibroblasts tikus), limfosit tikus dan limfosit pinggiran manusia. Kajian selanjutnya telah dijalankan untuk memperhatikan perubahan morfologi dalam sel WEHI 3B yang dirawat dengan menggunakan cahaya, pancaran dan imbasan elektron mikroskop. Sifat morfologi, apoptosis dan nekrosis juga diperiksa dengan mikroskop pendarfluor di mana sel diwarnai dengan akridin oren (AO) dan propidium iodida (PI). Sel yang dirawat dengan NDV strain AF 2240 dan V4-UPM menunjukkan sifat apototik seperti kecutan sel, blebbing sel, pembentukan jasad apototik dan sel nekrotik berbanding dengan sel pengawal negatif tidak menunjukkan sebarang sifat apoptosis dan nekrosis. Apoptosis awal juga diperhatikan melalui mikroskop pendarfluor di mana sel telah diwarnakan dengan Annexin V dan PI.

Kesan virus terhadap pembelahan sel telah dikenal pasti dengan teknik MTT dan BrdU. Di samping itu, dari segi molekul, kedua-dua strain virus ini dapat menyebabkan pemotongan DNA internukleus untuk menghasilkan pelbagai serpihan 180-200 bp yang dapat dilihat sebagai tangga pada gel agarosa. Awal apoptosis juga diperhatikan melalui Annexin V kaedah aliran sitometri. Peratus apoptosis untuk sel WEHI 3B yang dirawat dengan NDV strain AF2240 dan V4-UPM telah meningkat dari semasa ke semasa. Kitar sel dan apoptosis juga ditentukan melalui kaedah aliran sitometri PI. Kedua-dua strain NDV tidak dapat menahankan WEHI 3B pada fasa kitar sel yang tertentu dengan menggunakan kaedah aliran sitometri PI.



Dalam kajian ini aktiviti anti-leukemik pada kedua-dua strain NDV telah dikaji dengan tikus BALB/c yang telah dicetuskan leukemik dengan sel WEHI 3B. Pada hari keesokan mereka telah dirawat dengan NDV strains AF2240 dan V4-UPM dan arabinositosin sebagai ubat kormesial yang dijadikan pengawal positif. Kumpulan tikus yang dirawat dengan arabinositosin, NDV strain AF 2240 dan V4-UPM, masing-masing menunjukkan kesan pembunuhan yang ketara ($p < 0.05$) terhadap sel leukemic berbanding dengan tikus untuk kumpulan tanpa sebarang rawatan. Jumlah sel darah putih dan peratus sel blast di sapuan darah, sum-sum tulang dan limpa adalah rendah ($p < 0.05$) di tikus yang dirawat dengan NDV strain AF 2240 dan V4-UPM, masing-masing, berbanding dengan kumpulan tikus tanpa rawatan yang menunjukkan nombor leukimia yang tinggi. Keberatan limpa dan hati adalah rendah secara ketara ($p < 0.05$) di kumpulan tikus yang dirawat dengan arabinocytocine, NDV strain AF 2240 dan V4-UPM, masing-masing, berbanding dengan kumpulan tikus tanpa sebarang rawatan yang menunjukkan splenomegaly dan hepatomegaly yang ketara ($p < 0.05$).

Kajian histopatologikal juga dijalankan dan mengesahkan keputusan hematologikal. Daripada keputusan yang diperolehi, kumpulan tikus yang dirawat dengan kedua-dua NDV strain AF 2240 dan V4-UPM menunjukkan keputusan yang seiras dengan arabinocytocine yang merupakan ubat kormesial untuk leukemia. Pewarna immunoperoxidase, analisa hemagglutinin dan real time PCR telah dijalankan untuk mengesan NDV di organ tikus setelah dirawat dengan NDV. Keputusan telah menunjukkan tiada zarah NDV dikesan di organ tikus. Kajian ini menunjukkan NDV



strain AF 2240 dan V4-UPM menyebabkan kesan sitolitik terhadap sel leukemia WEHI 3B secara *in vitro* dan *in vivo*.



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I certify that a Examination Committee has met on 22 September 2008 to conduct the final examination of Aied Mohammed Al-Absi on his Doctor of Philosophy thesis entitled “Cytolytic Effects and Apoptosis Induction of Newcastle Disease Virus Strains AF 2240 and V4-UPM on Leukemia Cell Lines In vitro and In vivo” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy. Members of the examination committee were as following:

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously and is not concurrently submitted for any other degree at UPM or at any other institutions.

AIED MOHAMMED AL-ABSI

Date: 26 February 2009



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