

# Studies of the antiviral activity of the Dichloro flavon against Polio virus and Rubella Virus in tissue culture

Abdul latif Mohamed Ahmad<sup>1</sup>, Mohamed Omar Abdul latif<sup>2</sup>

<sup>1</sup> Collage of Medicine, Hawler Medical University,

<sup>2</sup> Biotechnology research Center, Al-Nahrain University

( Received 1 / 6 / 2009 , Accepted 25 / 10 / 2009 )

## Abstract:

Flavone originally founded in herbs and plants such as beans, tomato and grapefruit.

Dichloro flavan relatively non-toxic, we find in this study that when we test the toxicity of this compound in the following tissue culture (HeLa, WISH, RD and L20B) and we find also that the toxic concentration between 16-32 µg/ml. The concentration 0.06 µg/ml inhibit (100TCID<sub>50</sub>) of poliovirus in RD cells and L20B cells.

used the Therapeutic Index (TI) which is the ratio between the toxic concentrations of the drug over the minimal inhibitory concentration. If the TI one or less means that the compound not advise to use due to the high side effect while if the TI more than one compound maybe used for treatment.

The TI of the compound against Polio virus more than 100

This means that the compound in case of used as treatment in human has no any side effect.

## Introduction

The antiviral agent could be said to have been born in 1951 with the discovery that certain thiosemicarbazones were effective in the treatment of vaccinia virus infection. However, the story of real and active antiviral agents began in 1962 with the introduction of iodoxuridine (IUDR) [1,2]. The last few decades have seen dramatic advances in the range and effectiveness of antibiotics and chemotherapeutic agents for treatment of bacterial infection. The development of drugs with effective antiviral activity has proved much more difficult .the root of the problem lies in the nature of viruses and the way in which they damage the cells [3]. There is another problem which is that symptoms of disease usually occur only after substantial virus replication within cells has already occurred. Such cells are damaged or destroyed by the invading virus, and symptoms occurred as a result of this damage. Antiviral treatment at this stage is ineffective and the majority of infections unnecessary, for by this time the host's own immune defenses have been primed to limit further spread of virus, and recovery follows as damaged cells regenerate. To be effective, therefore, treatment must be instituted early in the infection, or be given prophylactically[4]. Under these circumstances the identity of the invading virus is not usually known, only compounds with broad – spectrum antiviral activity and low toxicity are appropriate. Furthermore, the cost of developing a new chemotherapeutic agent has increased enormously, since many agencies alarmed by the thalidomide (a sedative drug found in 1961 to have caused malformation of the limbs in babies whose mother took it during pregnancy) disaster, began in 1962 to demand amore comprehensive evidence of lack of teratogenicity and carcinogenicity as well as toxigenicity in man.

For these reasons, antiviral therapy for majority of virus infections is not feasible, nor in many instances it desirable.

the objective of this study is to search for effective antiviral compound for more than one viral families, we chose poliovirus which is non enveloped RNA belong to the family picornaviridae and rubella virus which is an enveloped RNA belong to the family togoviridae [5, 6].

## Material and methods

### Cells (from Biotechnology research Center, Al-Nahrain University )

- WISH cells were grown at 37° C in MEM (Gibco) supplemented with 10% fetal bovine serum (FBS).
- HeLa cells were grown at 37° C in MEM supplemented with 10% FBS.
- RD cells were grown at 37 ° C in EME supplemented with 10% fetal calf serum (FCS).
- L20B cells were grown at 37 ° C in EME supplemented with 10% FCS to all media during culture the following agent added (penicillin, Streptomycin and glutamine).

### Viruses (Vaccine)

- Laboratory passaged strain of poliovirus grown in L20B cells monolayer maintained in EME supplemented with 2% FBS.
- Laboratory passaged strain of rubella virus grown in chick embryo fibroblast cells monolayer maintained in MEM supplemented with 2% FCS Cultures were harvested at full cytopathic effect (CPE) frozen and thawed clarified by centrifugation and the suspension was stored at -70° C.

### Antiviral agent

Dichloro flavon (DCF) was supplied by Wellcome Research Laboratory, Kent ,UK.The drug obtained in powder form and dissolved in dimethyle sulfoxide (DMSO)

(Sigma chemical) then stock solution were stored at 4 ° C The determination of therapeutic index. Therapeutic index (TI) which is ratio of the dose of the drug which is just toxic (Maximum tolerated dose) to the dose which is just effective (Minimum effective dose). If this index is one or less it is not possible to use the drug under the conditions out lined without causing side effect, but if this index is larger than the margin of safety is accordingly great.

## Results

### *Studies in tissue culture toxicity*

The toxicity of the antiviral compound was assessed in HeLa cells, WISH cells, RD cells and L20B cells, by inspection of monolayers maintained for 5 days in media with various concentrations of the compound. In HeLa

cells, WISH cells and RD cells 16 µg/ml. induced morphological changes or cell death, but the effect of the compound on L20B is 32µg/ml.

#### **Inhibition of CPE by the DCF**

Serial 2-fold dilutions of the compound were made starting just below the toxic concentration. These were added with virus to the wells of 96 well microtitre plates containing confluent monolayers of HeLa cells, WISH cells, RD cells or L20B cells. They were observed for CPE daily for 5 days. The minimal inhibitory concentration (MIC) of the drug was calculated according to Karber as 50% end-point.

The MIC of the compound were 0.6 µg/ml against poliovirus in both cell culture the RD, and the L20B cells, while the drug has no effect against rubella virus (Table 1)

**TABLE 1**

Cell system	Type of virus	MIC	MTC*	TI
HeLa	Rubella	> 16	16	<1
WISH	Rubella	> 16	16	<1
RD	Polio	0.03	16	54
L20B	Polio	0.03	32	108

MICs and minimal toxic concentrations (µg/ml) of the DCF in different cell systems

\*For the toxicity test the drug concentrations added to the cells without virus.

#### **Reduction of viral yield by the DCF**

studied the yield of virus in the presence of selective concentrations of the compound against poliovirus in L20B cells. The yield was greatly reduced by the compound DCF (Table 2).

**TABLE 2**

DCF µg/ml	No. of plaque	Ratio of plaque formed untreated / treated
0	46	1
0.01	34	1.4

## **References**

- 1-Kaufman, H.E., Martola, F. and Dohiman, C (1962) the use of 5-iodo-2-deoxyuridine in the treatment of herpes simplex keratitis. Arch.Ophthalmol.N.Y.68:253-239
- 2- Kaufman, H.E., Varnell, E.D. and Sanitato, J.G (1984) Virus chemotherapy: antiviral drugs and interferon. Antiviral research 4:333-338
- 3-Jawetz, E., Melnick, J. and Adelberg, E.A :Brooks, G., Bulet, J. and Morse, S. (2004), Medical Microbiology , 23<sup>rd</sup>. ed., LANCE.
- 4-Ahmad, A.L.M. and Tyrell, D.A.J .(1986): synergism between anti-rhinovirus antiviral. Antiviral research. 6: 241-250
- 5-Freg, T.K. and Wolinsky, J.S.(1999) Rubella virus. In encyclopedia of virology, 2<sup>nd</sup>. Ed. Academic press 265-276.
- 6-Champoux, J.J.(2004): Biology of viruses .In: Sherris, J.C., Sherris Medical Microbiology, an introduction to Infectious diseases , 4<sup>th</sup>. Ed., McGraw-Hill pp:77-112
- 7-Graham, J.:(1967) Pharmacology for medical student 1<sup>st</sup>. Ed.
- 8-Paolacci, A.R., Ovidio, R.D. and Marabottini, R.(2001): Induces a differentiation accumulation of phenylalanine

0.03	3	15.3
0.06	0	( not applicable)

Activity of DCF against poliovirus demonstrated by plaque reduction in L20B cells

## **Discussion**

In spite of relatively extensive efforts to develop antiviral drugs .the evaluation of antiviral compound can be quantitated by using the therapeutic index [7].

The present study upon the toxicity of DCF confirms that this compound is relatively non-toxic substances with an inhibitory effect against poliovirus in RD cells and L20B. in fact the flavonoid compounds are generally found in many plants and herbs such as tomatoes, beans and grape fruits [8, 9]

Since the inhibition of CPE is relatively simple test it has some value for preliminary experiments to evaluate antiviral drugs.

DCF fail to inhibit rubella virus may be due to the fact that this virus is an enveloped virus [10]. Toxicity of the compound DCF to L20B is slightly higher than other three types of cell cultures may be due to that all these three cell cultures of human Oregon while the L20B cells of mice Oregon [11, 12].

It seemed probable that a more sensitive test based on plaque titration may have given more accurate and reproducible results. However poliovirus plaques were inhibited by nearly the same concentration of DCF as was required to inhibit 100 TCID<sub>50</sub> in L20B cells , the L20B cells is more sensitive to poliovirus than RD cells [13].

When use the TI for drug against rubella virus find that this index is very low therefore rubella virus is resistant to this drug while this index is more than 100 when the drug used against poliovirus. Therefore DCF if used in man with no side effect and we recommend to use this compound since it is non toxic and with very low cost in addition that the flavonoid compounds have the ability to transport across blood-brain barrier [14].

ammonium logase chalcone in sensitive and resistant bean cultivars. J. of plant physiology 2: 28-34

9-Verhogen, M.E., Bovy, A., Collins, G. and Colliver, S.(2002): increasing antioxidant levels in tomatoes through modification of flavonoid biosynthesis pathway. J.of exp. Botany 53(377) 2099-2106

10-Jameil, Z.J.(2007): isolation and characterization of poliovirus from vaccinated children with poliovaccin : Thesis Department of biology college of sciences, university of Baghdad

11- Koller, M.R., Palsson, B.O. and Master, J.R. (2001): Human cell culture (vol.5) Kluwer Acad. Publishers, London, pp 241.

12-Crotty, S., Saleh ,M., Gitlion, L. Beske, O. and Andino, R.(2004): The poliovirus replication machinery can escape inhibition by an antiviral drug that targets a host cell protein , J. of virology 78(7)3378-3386

13-Freshney, R.I.(2000) : Culture of animal cells: A manual of basic technique (4<sup>th</sup>. Ed.). Inc. publication New York, USA.

14-Mitsunaga, Y. Takanaga, H. Natio, M. and Sawada, Y.  
(2007) : effect of bioflavonoids on vincristine transport  
across blood-brain barrier J. of antimicro. chemotherapy.

## دراسة فعالية المركب دايكلوروفلافين المضادة لنمو فايروسات شلل الاطفال والحصبة الالمانية في الزرع النسيجي

عبد اللطيف محمد احمد<sup>١</sup>، محمد عمر عبد اللطيف<sup>٢</sup>

<sup>١</sup> كلية الطب، جامعة هوليير الطبية، اربيل، العراق

<sup>٢</sup> مركز بحوث التقانة الاحيائية، جامعة النهرين، بغداد، العراق

( تاريخ الاستلام: ١ / ٦ / ٢٠٠٩، تاريخ القبول: ٢٥ / ١٠ / ٢٠٠٩ )

### الملخص:

يعد مركب الفلافون من المركبات الموجودة في عدد من الاعشاب والنباتات الطبيه مثل الفاصوليا والطماطه والكريب فروت. ان مركب الدايكلوروفلافين غير سام نسبيا فعندما تم دراسته تاثيراتها السمية على خلايا الزرع النسيجي المستعمله في هذه الدراسه وهي HeLa, WISH, RD and L20B وجد ان التراكيز السامه لمركب الدايكلوروفلافين تتراوح بين ١٦ µg/ml الى ٣٢ µg/ml بينما التركيز ٠,٠٦ µg/ml يمنع نمو (100TCID50) لفايروس شلل الاطفال في خلايا الزرع النسيجي RD and L20B بينما فشل التركيز ٣٢ µg/ml للماده لمنع فايروس الحصبة الالمانية في خلايا HeLa, WISH. للحكم على ان الماده ليس لها تاثير جانبي على الخلايا استخدم في هذه الدراسه الدليل العلاجي (TI) Therapeutic Index هو عبارته عن نسبه التركيز السمي للماده على التركيز الاقل الذي يمنع النمو. فأذا كان الرقم يساوي ١ فما دون، تكون الماده غير صالحه للاستخدام واذا كان هذا الرقم كبيرا فيعني ان هذا المركب قليل او عديم الاصابات الجانبية . ان الدليل العلاجي لهذه الماده عند استخدامها ضد فايروس شلل الاطفال كان اكثر من ١٠٠ وهذا يعني ان هذا المركب يصلح استخدامه كعلاج للانسان دون تاثيرات جانبية تذكر . اجري البحث في مركز بحوث التقانة الاحيائية /جامعة النهرين ومن نفس المركز تم الحصول على خطوط الخلايا اما الفيروسات فهي عبارة عن لقاح تم الحصول عليه من المراكز الصحية في بغداد.