Studies of the antiviral activity of the Dichloroflavon against Polio virus and Rubella Virus in tissue culture

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

Abstract:
Flavone originally founded in herbs and plants such as beans, tomato and grapefruit. Dicholoroflavon 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 (100TCID50) of poliovirus in RD cells and L20B cells.

Introduction
The antiviral agent could be said to have been born in 1951 with the discovery that certain thiospermicarbazones 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 37º C in MEM (Gibco) supplemented with 10% fetal bovine serum (FBS).
- HeLa cells were grown at 37º C in MEM supplemented with 10% FBS.
- RD cells were grown at 37 º C in EME supplemented with 10% fetal calf serum (FCS).
- L20B cells were grown at 37 º 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-70º C.

Antiviral agent
Dichloroflavon (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 º 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 f 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

<table>
<thead>
<tr>
<th>Cell system</th>
<th>Type of virus</th>
<th>MIC</th>
<th>MTC*</th>
<th>TI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>Rubella</td>
<td>&gt;16</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>WISH</td>
<td>Rubella</td>
<td>&gt;16</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>RD</td>
<td>Polio</td>
<td>0.03</td>
<td>16</td>
<td>54</td>
</tr>
<tr>
<td>L20B</td>
<td>Polio</td>
<td>0.03</td>
<td>32</td>
<td>108</td>
</tr>
</tbody>
</table>

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 agent poliovirus in L20B cells. The yield was greatly reduced by the compound DCF (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>DCF µg/ml</th>
<th>No. of plaque</th>
<th>Ratio of plaque formed untreated / treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>0.01</td>
<td>34</td>
<td>1.4</td>
</tr>
</tbody>
</table>

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 organically 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 TCID50 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 coast in addition that the flavonoid compounds have the ability to transport across blood-brain barrier [14].

**References**


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

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

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الملخص:

بعد مركب الفلافون من المركبات الموجوده في عدد من الاعشاب والنباتات الطبيه مثل الفاصوليا والطماطه والكريب فروت، ان مركب الدايكلوروفلافين غير سميا فعندما تم دراسة تاثيراتها السمية على خلايا الزرع النسيجي المستعمله في هذه الدراسه وهي HeLa, WISH, RD and L20B وجد ان التراكيز السامه لمركب الدايكلوروفلافين تتراوح بين 24 μg/ml إلي 33 μg/ml بينما RD and L20B بينما فشل التركيز 0.6 μg/ml لمنع نمو (100TCID50) فايروس شلل الاطفال في خلايا الزرع النسيجي في خلايا RD and L20B. لتحكم على ان المادة ليس لها تاثير جانبي على الخلايا استخدم في هذه الدراسه الدليل العلاجي (THERAPEUTIC INDEX) TI هو عبارة عن نسبة التركيز السمي للمادة علي التركيز الاقل الذي يمنع النمو. فأذا كان الرقم يساوي 1 فما دون، تكون المادة غير صالحة للاستخدام. إذا كان هذا الرقم كبيرا فمعنى ان هذا المركب قليل أو عدم الإصابات الجانبية. ان الدليل العلاجي لهذه المادة عند استخدامها ضد فايروس شلل الاطفال كان أكثر من 100 وهذا يعني ان هذا المركب يصلح استخدام كعلاج للإنسان دون تأثيرات جانبية تذكر. اجري البحث في مركز بحوث التقانة الاحيائية / جامعة النهرين ومن نفس المركز تم الحصول على خلايا ام الفيرونا في بغداد.

عبارة عن نفاذا تجص على من المراكز الصحية في بغداد.