The antiviral activity of the compound chalcone (4-ethoxy-2-hydroxy-4, 6-dimethoxy-chalcone) against rubella virus *in vitro*

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Abstract:

The studies on the antiviral compound chalcone *in vitro* in both tissue and organ culture systems against rubella virus glass that this compound relatively non toxic to the cell culture and organ culture of the concentration of 8 ug/ml or less, chalcone have significantly antiviral activity against rubella virus in tissue culture and organ culture. We find that a concentration of 0.03ug/ml or more inhibit the IOOTCID50 of rubella virus. The therapeutic index (TI) used in this study to evaluate the drug, the (TI) which is the ratio of the dose of 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 not possible to use the drug under the conditions outlined without causing side effect, if the index is larger than the margin of safety is accordingly great, the TI of chalcone against rubella virus more than 70, therefore this compound if used in man have no side effect .

Introduction

Rubella is a mild infection without significant sequel as, which would barely be worth prevention or treatment were it not for its teratogenic effects in pregnancy. Children with congenital defects following maternal rubella of which deafness, blindness, heart disease, psychomotor retardation and death were consequence(1).Live vaccines which are safe and effective are available and young children have reduced the incidence of congenital rubella, the vaccine as it was mentioned reduced the incidence of rubella and therefore have presented as candidates for antiviral therapy however significant additional number of at risk pregnancies are terminated. There is clearly at present a limited but individually important need for anti-rubella virus, chemotherapy, (2) nevertheless the development of compounds specifically for use in pregnancy would present some difficulties. The need for antiviral drugs is directly depends on the clinical importance and prevalence of virus infections on the availability, safety, effectiveness, acceptability for prophylaxis and therapeutic antiviral use agent (3).In present study, chalcone which is related to an antiviral flavone originally isolated from several herbs and plants such as agastache folium, beans, tomato, grapefruit and many other plants and herbs (5,6,7).These flavones have the ability even to across the blood-brain barrier (5,6) which is a highly specific and potent inhibitor of cold virus *in vitro* (8,9,10, 11,12).We considered it appropriate to evaluate the anti rubella virus activity in tissue culture and in organ culture.

Material and Methods Cell

1.Human cervical carcinoma cells, Hela cells are epithelial like cells derived from human epithelial cervical carcinoma.The cells were propagated and maintained in Eagles Minimum Essential Medium (MEM) (Gibco) supplemented with 10% fetal calf serum (FCS) as a growth medium or 2% FCS as a maintenance medium.

2. Human diploidjlung cells (MRC-5):

MRC-5 is fibroblastic human diploid lung cells. The cells were grown in similar manner to Hela cells.

3. Chick Embryo Fibroblast (CEF)

CEF are fibroblastic chick normal cells derived from 10- day normal chick embryo the cellswere grown in MEM supplements with 10% FCS as growth medium or 2% FCS asmaintenance medium.

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4.Chick embryo tracheal epithelial (CETE) organ culture.

Trachea was washed thoroughly with organ culture medium (MEM supplemented with 1% Bovine Plasma Albumin (BPA).The con-nective tissue surrounding the trachea was removed, and then the trachea was cut trans- versely into rings (2-5 mm) and placed in test tubes containing 1 ml of organ culture medium (one ring per tube). The culture was incubated at 33°C and beating of cilia was observed daily by microscope before and during the experiment.

Virus Titration

Virus infectivity was assayed by titration in microtiter plates with confluent monolayer of Hela, MRC-5 or CEF and in CETC using half log dilution and 3 or 4 wells perdilution in tissue culture and 2 tubes perdilution in CETC,50% end points were calculated according to Reed and Mounch (1942). The virus infectivity was measured by CPE or hemadsorbtion in tissue culture and by ciliary beating in organ culture.

Chemicals

The antiviral drug chalcone (4-ethoxy-2-hydroxy-4,6-dimethoxy-chalcone) (C19H19O5) was supplied by Nippon-Roche.

Rubella virus

Rubella virus was grown in CEF cells monolayer, cultured in BME supplemented with 5% FCS.Culture were harvested at max-imum CPE (48 hours) Frozen and thawed, clarified by centrifugation and supernatant stored at -70°C.

Hemadsorption Test

0.5% of guinea pig RBC was added to the infected tissue culture, after 3-5 minutes incubation the agglutination was observed.

Results

Toxicity of chalcone was estimated by incorporating varying amounts of the drug (0.5-64ug/ml) in the maintenance medium of tissue culture or in organ culture medium examining the cells daily for toxic effect such as floating cells, cell granulation or any alteration of the cells, same examination to the organ culture by observation of ciliary beating.

Table (1). Cytotoxic effect of chalcone:

CETE	CEF	MRC-5	Hela cells	Cone, of ch. ug/ml
T	T	T	T*	64
T	T	T	T	32
T	T	T	T	16
T	T	NT	NT*	8
NT	NT	NT	NT	4
NT	NT	NT	NT	2
NT	NT	NT	NT	1
NT	NT	NT	NT	0.5

T*= Toxic
NT*=Non Toxic

It was found (Table 1) that a concentration of 16 ug/ml or greater were rapidly toxic for tissue culture and organ culture.

The determination of minimal inhibitory concentration (MIC) of chalcone were determined by serial two-fold dilution of the drug starting just below the toxic concent-ration of chalcone to the tissue culture and organ culture(0.4ug/ml).These concentrations of the drug were added together with 100TCID50 of rubella virus to the wells of 96 wells microtiter plate containing confluent monolayer of Hela, MRC-5, CEF, the drug also added to the organ culture CETE. They were then observed daily for CPE and for heamagglutination (one drop of the media plus one drop of 5% guinea pig RBC), in tissue culture and observation of ciliary beating for CETE for five days. The media from infected tissue culture and organ culture were titrated in tissue culture of Hela cells. Table(2) showed that no CPE or hemadroption at concentration of chalcone at 0.5 ug/ml or greater while CPE or hemadr-option observed at concentration of 0.06 ug/ml or lower.

Table (2).potency of chalcone against 100 TCLD50

Ciliary beating of CET	CPE/ Hemadsorbtion			Concentration of drug ug/ml
	CEF	MRC-5	Hela	
CB	—	—	—	4
CB	—	—	—	2
CB	—	—	—	1
CB	—	—	—	0.5
CB	++/HA	++/HA	+	0.125
NB	+++/HA	+++/HA	++/HA	0.06
NB	++++/HA	++++/HA	++++/HA	0.03
NB	++++/HA	++++/HA	++++/HA	0.01
NB	++++/HA	++++/HA	++++/HA	0.00

- — No CPE, No hemadsorbtion
- + 25% CPE
- ++ 50% CPE
- +++ 75% CPE
- CB= Ciliary Beating
- NB= Non Ciliary Beating

The yield of virus were also studied Fig (1) showed that no virus detected at the presence of drug at concentration of 0.06 ug/ml or greater while virus titer was 10^7 when the drug at concentration of 0.03 ug/ml or none.

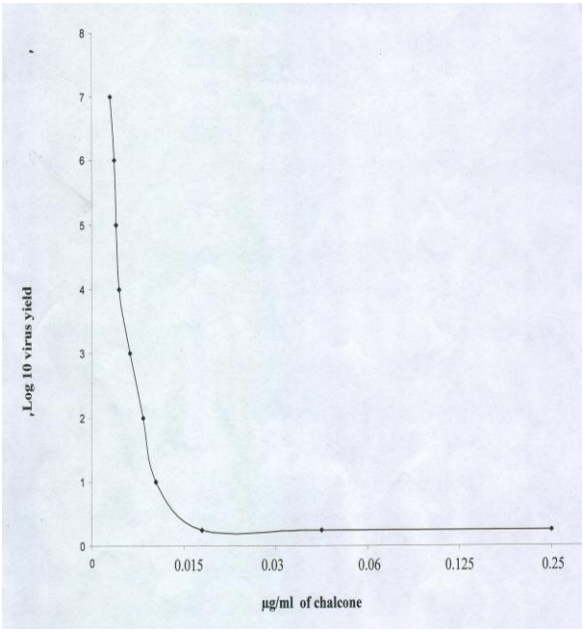

Fig l: yield of virus treated with chalcone

Discussion

In the present study, on the antiviral activity of chalcone against rubella virus. The toxicity of chalcone was first studied in tissue culture of Hela cells, MRC-5 cells and CEF cells in addition to organ culture CETE starting from concentration of (0.5 ug/ml) the toxicconcentration of the drug were 16 ug/ml and over while a concentration of 4 ug/ml and lower were non toxic in both systems (organ culture, tissue culture).

It therefore seem likely that concentrations of drug used to test the antiviral activity must be started at concentration just below the toxic concentration i.e. at concentration of 4 ug/ml and lower.The minimal inhibitory concentration of the drug (MIC) in tissue culture system and organculture were 0.125 ug/ml and 0.06 ug/ml respectively therefore the therapeutic index (TI). [Is the ratio of the drug concentration that is just toxic to the tissue culture (Maximum tolerated dose MTD) to the minimum inhibitory concentration (MIC) of the drug]. If this index is one or less it not possible to use the drug under the conditions outlined without causing side effect, if the index is larger than the margin of safety is accordingly great) (13,14). In this study the (TI) is more than 100 these results conclude that chalcone is relatively non toxic. We use organ culture system in the assay of the activity of chalcone because the antiviral activity proved that organ culture very convenience since it resembles natural infection. Indeed Bucknall indicated that 70% of compounds active against influenza virus in tissue culture failed to inhibit viral multiplication at nontoxic level in organ culture of ferret trachea.

In addition to use organ culture in our study for the antiviral activity we used two test system to prove that the antiviral activity is real, the use of hemadroption test with 5% guinea pig RBC and the virus yield from all tissue culture and organ culture treated with chalcone and infected with 100 **$TCID_{50}$,** indeed all these results fined that chalcone had an anti rubella virus activity, our final conclusion that chalcone seem to have beneficial effect in man infected with rubella since it is nontoxic.

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فعالية المركب جالكون المضادة لنمو فايروس الحصبة الالمانية في الزرع النسيجي والزرع العضوي

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الخلاصة

في هذه الدراسة تم دراسة الفعالية المضادة لفايروس الحصبة الالمانية لمادة الجالكون مختبريا باستخدام النظامين المعروفين وهما الزرع النسيجي والزرع العضوي وجد ان هذه المادة غير سامة للزرع النسيجي والزرع العضوي اذا استخدمت بتركيز (8 مايكروغرام/ مل) او اقل كما وان هذه المادة لها تاثير واضح جدا مضاد لفايروس الحصبة الالمانية في الزرع النسيجي والزرع العضوي . فقد وجد ان 0.03 مايكروغرام او اكثر لا تسمح بنمو (100TCID50) لفايروس الحصبة الالمانية وباستخدام الميزان العلاجي(TI) والذي هو نسبة اقل تركيز للمضاد الفايروسي بسبب تسمم للخلايا الى اقل تركيز للمضاد الفايروسي الذي يسبب منع نمو (100TCID50) للفايروس حيث اذا كان هذا الميزان ان (TI) واحد اقل فلا يسمح باستخدام المضاد الفايروسي حيث ان له تاثيرات جانبية كبيرة واذا ما كان اكثر من واحد فانه يصلح للاستخدام وكلما زاد الرقم كلما قلت التاثيرات الجانبية او انعدمت ففي دراساتنا هذه وجد ان (TI) اكثر من (70) لهذا فان هذه المادة في حالة استخدامها في الانسان ليسي لها اي تاثيرات جانبية .