The In Vitro Inhibitory Effect of Flavonoid Astilbin on 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase on Vero Cells

**Background.** Epidemiological studies have shown that hypercholesterolemia is a major risk factor for coronary heart disease. Clinical trials of lipid lowering therapy, 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase inhibitor has been shown to decrease cardiac events and mortality. Flavonoids are polyphenolic natural antioxidants existing in vegetables, fruits and beverages such as tea and wine. Previous studies have shown that some antioxidants had hypocholesterolemic effect, and flavonoids in take was associated with the decrease of mortality from coronary artery disease. The aim of this study was to evaluate the inhibitory effect of flavonoids on HMG-CoA reductase.

**Methods.** The methods for analyzing specific inhibitors of mevalonate biosynthesis have been well-established, using Vero cells, a cell line obtained from kidneys of African green monkeys. Flavonoids isolated from different traditional Chinese herbs were dissolved in DMSO and incubated with Vero cells with or without the addition of 1 mM mevalonate or 5 mM sodium acetate in order to observe cell growth for 24 h.

**Results.** Concentrations of 1 mM mevalonate or 5 mM sodium acetate were added into culture medium in order to observe the effect on cell growth. Different concentrations of pravastatin to inhibit cell growth were used as a positive control. About 40 flavonoid compounds were used for study, only one compound, astilbin (belonging to the flavonol group), showed significant inhibition of Vero cell growth.

**Conclusions.** This study shows that one flavonoid compound, isolated from traditional medicinal herbs, may be an effective HMG-CoA reductase inhibitor which might be developed into a new hypocholesterolemic agent. [Chin Med J (Taipei) 2001; 64:382-387]

---

Flavonoids are a large group of polyphenolic compounds possessing antioxidant activity that occur naturally in a variety of foods from vegetables or in such as apples, on ions and beverages such as tea and red wine. The most important groups of flavonoids are anthocyanins, flavonols, flavones and flavonones. Flavonols are scavengers of superoxide anions, singlet oxygen, and lipid peroxyl radicals, and they can sequester metal ions through liganding. Oxidized low-density lipoproteins Ox-LDL are cytotoxic and atherogenic, and are believed to be an important step in the formation of atherosclerotic plaques. A pre vi-

---

Received: July 18, 2000. Accepted: April 16, 2001.
Correspondence to: Paul Chan, MD, PhD, Division of Cardiovascular Medicine, Taipei Medical University-Wan Fang Hospital, 111, Sec. 3, Hsin-Lung Road, Taipei 117, Taiwan. Fax: 886-2-2933-9378; E-mail: chanpaul@wanfang.gov.tw
ous report showed that flavonols and flavones had anti platelet effect and reduced thrombogenic ten
dcies, and the mech a nism was prob a bly due to in hi tion of cyclo-oxygenase. Flavonoids have also been
stud ied in re la tion to their im prove ment of vas cu lar
fragility.

Multiple epidemiological stud ies have shown that in creased veg etable and fruit con sump tion has in
verse re la tion ship to the oc cur rence of stroke and
CHD in differ ent pop u la tions. The ex pla na tion of this phe
mon e non is prob a bly due to an in crease of
flavonoid in take. For ex am ple, the Zutphen Study has shown that flavonoid in take was sig nif i cantly in
versely as so ci ated with mor tal ity from CHD and
showed an in verse re la tion with in ci dence of myo-
car dial in farc tion. The rel a tive risk of cor o nary
heart dis ease mortality in the high est ver sus the low-
est tertile of flavonoid in take was 0.42. Besides
be nef i cial an ti ox i dant ef fect of flavonoid, we pre-
sume that the positive results of the above men tion ed ep i de mi o log i cal stud ies were prob a bly due to hypocholesterolemic ef fect. Pre vi ous stud ies have
dem on strated that some an ti ox i dants, such as
beta-carotene, vitamin C and vitamin E have
hypocholesterolemic ef fect, which is re lated to the
in hi bi tion of HMG-CoA reductase. This study
was un der taken to eval u ate whether flavonoids have
HMG-CoA reductase in hib itory ef fect.

**Methods**

Flavonoid com pounds (flavonols, flavones, flav-
ones) were iso lated in the lab or atory of Dr. Kuo of
China Med i cal Col lege, Tai-Chung. Flavonoid com-
po nds were dis solved in 100% DMSO. Cell cul ture me di um had 0.01% DMSO (cells 3 × 10^4). After in cu ba tion for 24 h, cell growth and mor phol o gy
was ex a men ed by light mi cro scope (Olympus, Japan). Con cen tra tions of 1 mM mevalonate or 5 mM sodium
ac e tate were added into cul ture me di um in or der to
ob serve the ef fect on cell growth. Differ ent con cen tra-
tions of pravastatin to in hibit cell growth were used as
a posi tive control.

**Vero cells**

Vero cells, an es tab lished cell line from kid ney
cells of Af ri can green mon key, were grown in a hu-
mid i fied in cu ba tor (5% CO_2) at 37 °C in a 1-1 glass
flask con tain ing 200 ml of Eagle’s min i mum es sen-
tial me di um (MEM) sup pli ed with pen i cin (100 units/ml), strep to my cin (100 µg/ml), 0.075%
(w/v) NaHCO_3, 0.03% (w/v) glutamine, and calf se-
rum at 5% (v/v) (5% CS-MEM). Cells were
sub cul tured ac cord ing to stand ard try sin ization pro-
ce dures. Vero cells (3 × 10^4 cells) in 100 µl of the
above mix ture, but now with calf se rum at 2% (v/v),
were in oc u lated in each well of microplates and in cu-
bated at 37 °C for 1 h. Then, vari ou s amounts of
flavonoid com pounds (flavonols, flavones, flavonones)
100 µg/ml with and with out 1 mM mevalonate or 5
mM so di um ac e tate were added to the wells. Vero
cells were grown in a hu mid i fied in cu ba tor (5% CO_2)
at 37 °C for 24 h.

**Measurement of cell growth**

Cell growth was mea sured by the method of
Armstrong. Cells grown on each well of 96-well
microplates were washed twice with 100 µl of cal-
cium- and mag ne si um-free phos phate-buffered sa-
line (PBS) and stained with 50 µl of stain ing so lu-
tion (methy losaniline 0.5%, NaCl 0.85%, for-
mamide 5% and eth a nol 50%) for 20 min utes. Then
the stain ing solution was re moved and the cells
were washed with wa ter. The absorbance at 540 nm
was mea sured by micro plate pho to me ter (Titertek
Co., Osaka, Ja pan).
Results

Concentrations of cells

Vero cells were seeded in each well of a 96-well microplate at $1.0 \sim 6.0 \times 10^4$ cells in 100 µl of 2% CS-MEM. Confluent growth was observed after a 24-hour incubation when the cells were seeded at $3.0 \sim 4.0 \times 10^4$ cells/well. Thus, a concentration of $3.0 \times 10^4$ cells/well was adopted in subsequent experiments.

Effect of various chemicals on growth of Vero cells

Fig. 1 shows the effect of pravastatin on growth of Vero cells. Pravastatin at the dose age of 100 µM inhibited cell growth completely. However, when 1 mM mevalonate was added to the culture medium, both morphological changes and growth inhibition were overcome and the cells grew normally. Addition of 5 mM sodium acetate had no effect. Morphological changes and growth inhibition of various flavonoid compounds on Vero cells were observed at a uniform dose of 100 µg/ml. These data indicated that this method was highly sensitive to mevalonate biosynthesis inhibitors.

The results indicated that most of the flavonoid compounds could not inhibit mevalonate biosynthesis in cultured cells (Tables 1-3). Although some flavonoid compounds had the inhibitory effect on growth of Vero cells, the effect was not over come after adding mevalonate into the culture medium. Only the astilbin (Table 1) showed potent inhibitory effect on growth of Vero cells, just like the effect of pravastatin.

Discussion

The Zutphen Elderly Study suggested that lower mortality from CHD and lower incidence of myocardial infarction was due to increased flavonoid intake. The pre dominant flavonoid in foods is quercetin.

| Table 1. Effects of flavonoids (flavonols) on the growth of Vero cells with or without mevalonate |
|----------------|----------------|-----------------|
| Compound       | Conc. (µg/ml) | Absorbance at 540 nm |
| Control        | 1% DMSO       | 0.837           |
| Galangin       | 100           | N               |
| Fisetin        | 100           | N               |
| Quercetin      | 100           | 0.323           |
| Quercitrin     | 100           | 0.104           |
| Rutin          | 100           | N               |
| Quercetin-3-O-(arabinopyanosyl)-galactopyranoside | 100 | 0.385 |
| Myricetin      | 100           | 0.576           |
| Myricitrin     | 100           | 0.392           |
| Kaempferol     | 100           | N               |
| Rhamnustrioside| 100           | N               |
| Astilbin       | 100           | 0.816           |

N = non-detectable.

| Table 2. Effects of flavonoids (flavanones) on the growth of Vero cells with or without mevalonate |
|----------------|----------------|-----------------|
| Compound        | Conc. (µg/ml) | Absorbance at 540 nm |
| Control         | 1% DMSO       | 0.187           |
| Naringenin      | 100           | N               |
| Naringin        | 100           | N               |
| Hesperetin      | 100           | 0.613           |
| Hesperidin      | 100           | N               |
| (+) Taxifolin   | 100           | N               |
| (+) Catechin    | 100           | N               |
| (-) Epicatechin | 100           | N               |

N = non-detectable.

Fig. 1. Effects of pravastatin (○) alone or in combination with 1 mM mevalonate (●) or 5 mM sodium acetate (Δ) on the growth of Vero cells.
cetin, and higher in take of quercetin re vealed in verse re la tion ship with CHD mor tal ity. The pres ent study also in cluded the com pound quercetin, but the re sults showed that only astilbin pos sessed sig nif i cant HMG-CoA reductase in hi bi tion.

It is pos si ble that quercetin and other flavonoids re duce the rate of for ma tion of oxi -
dised-LDL and thus in hibit the growth of ather o scle -
rotic plaques. In ad di tion, flavonoids in hibit cyclo-
oxygenases, which may re duce throm bo sis.

Hyper cho les ter ol emia con trib utes sub stan tially to the de vel op ment and clin i cal ex pres sion of cor o nary and other forms of ath ero scle ro sis.18-20 Con sider able ef fects of flavonoids on the growth of Vero cells with or without mevalonate

Table 3. Effects of flavonoids (flavones) on the growth of Vero cells with or without mevalonate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance at 540 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% DMSO</td>
<td>0.655 0.619</td>
</tr>
<tr>
<td>3-Hydroxyflavone</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>7-Hydroxyflavone</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Baicalin</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Chrysin</td>
<td>100</td>
<td>0.455 0.413</td>
</tr>
<tr>
<td>Apigenin</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Apigenin-7-O</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Luteolin-7-O-glucoside</td>
<td>100</td>
<td>0.371 0.395</td>
</tr>
<tr>
<td>Linarin</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Pectolinarin</td>
<td>100</td>
<td>N N</td>
</tr>
<tr>
<td>Cirsimarin</td>
<td>100</td>
<td>0.465 0.524</td>
</tr>
</tbody>
</table>

N = non-detectable.

References

1. Hertog MGL, Hollman PCH, Katan MB. Con tent of po ten-


15. Fuhrman B, El is A, Aviram M. Hypocho les terolemic ef feet of lycopene and beta-cartene is re lated to sup pres sion of cho lesterol syn the sis in the sis and augmen ta tion of LDL re ce ptor ac tiv ity in macro phages. *Biochem Biophys Res Commun* 1997;233:658-62.


