行政院國家科學委員會專題研究計畫 成果報告

藥物轉送 ☢☢☢基因多型性對於神經科常用藥物之影響

計畫類別：個別型計畫
計畫編號：anntttttttttttt
執行期間： 00年 00月 00日至 00年 00月 00日
執行單位：國立成功大學醫學系神經科

計畫主持人： 賴明亮
共同主持人： 黃金鼎
計畫參與人員：呂文仁、周真淩

報告類型：完整報告
處理方式：本計畫可公開查詢

中華民國 00年 00月 00日
行政院國家科學委員會補助專題研究計畫  ☑ 成果報告  □ 期中進度報告

藥物轉送 OATP-C 基因多行性對於神經科常用藥物之影響

Effects of polymorphism in OATP-C transporter over drugs commonly used in neurology

計畫類別：☑ 個別型計畫    □ 整合型計畫
計畫編號： NSC 93－2314－B－006－101－
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執行期間： 93 年 08 月 01 日至 95 年 07 月 31 日

計畫主持人：賴明亮
共同主持人：黃金鼎
計畫參與人員：呂文仁、周真凌

成果報告類型(依經費核定清單規定繳交)：□精簡報告    ☑完整報告

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□ 赴大陸地區出差或研習心得報告一份
□ 出席國際學術會議心得報告及發表之論文各一份
□ 國際合作研究計畫國外研究報告書一份

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管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：神經部

中華民國 95 年 09 月 05 日
I . Background

Drug transporters were expressed in different human tissue, including brain, intestine, liver, and kidney. They play key roles in the absorption, distribution, and excretion of the drugs and now known as one of the determining factors deciding drug effects. (Kim 2000, Kusuhara & Sugiyama 2002). Recently, many important transports have been cloned. In addition, significant progress have been made in characterizing the cellular properties and functions of such transporters (Gao & Meier 2001, Hirohashi et al 1999, Hirohashi et al 2000).

Among the transporters expressed at the basolateral membrane of hepatocytes, members of the organic anion transporting polypeptide (OATP) family have been shown capable of mediating the hepatic uptake of a variety of structurally divergent compounds. Within these, OATP-B, OATP-C, and OATP-8 have been established as the major OATPs located at the basolateral membrane of human hepatocyte.

OATP-C (genes SLC 21A6), also known as liver specific transporter-1 (LST-1) or OATP2, is involved in the hepatic uptake of a broad array of endogenous compounds, such as taurocholate (Hisang et al 1999) estrone sulfate (Tamai et al 2000), estradiol 17 β D- glucuronide, leukotriene C4, prostaglandin E2 and thyroid hormone (Abe et al 1999). OATP-C also is known to mediate the cellular uptake of bilirubin and its glucuronide conjugates. (Konig et al 2000, Cui et al 2001).

Ticlopidine, a drug currently used as a secondary prevention for ischemic stroke, which is a common disease seen in the aged people. We are interested in that whether combination of both medication causing a significant clinical effect due to interaction at transporter level.

Similarly, since both Hydergine® and Cerenin® are used in those patients who presenting early dementing syndrome and central or peripheral circulation dysfunction, the combined use of one together with ticlopidine will be frequently encountered in our clinical service. Since from our preliming data, all these drugs are related to OATP-C transporters, it’s likely drug-drug interaction may developed.

II . Goal of study

The goals of this two-year projects are

First year
1. To evaluate the SNP rate of OATP-C in normal Chinese people living at Taiwan
2. To study the effects of SNP for OATP-C of common neurological drugs in estradiol 17βD-glucuronide uptake in vitro (HEK-293 cell line)
3. To evaluate the pharmacokinetics of pravastatin in Chinese of various OATP-C genotypes.

Second year
1. To evaluate the pharmacokinetics of ticlopidine in Chinese with different OATP-C genotypes.
2. To study the drug-drug interaction in vivo between ticlopidine and Ergoloid Mesylate (Hydergine®) and Ginkgo Biloba (Cerenine®)
III. Methods

Research Methodology

First year

To evaluate the SNP rate of OATP-C in normal Chinese people living at Taiwan

a. Volunteers

We obtained blood sample from 120 healthy Chinese, unrelated people volunteers living at Tainan area through history, physical and laboratory tests results. The protocol was approved by IRB board at National Cheng Kung University Hospital.

b. Identification of Variants in OATP-C genes

The primer design was based on the published sequence (GenBank/ European Molecular Biology Laboratory accession NOS and No. AB026257 for the OATP-C genes) (Konig et al. 2000, Tamai et al. 2000). These primers create appropriate (approximate 350 bp) size for screening of polymorphism by subsequent single-strand confirmation polymorphism (SSCP). The subsequent methods have been described (Nishizato et al. 2003).

Briefly: After the PCR, the product (6UL) will be mixed with 3UL ethyl enediaminetetraacetic acid, formamide (95%), 0.05% bromphenol blue, heated at 95°C for 5 min and quick-chilled in a ice-bat. The single-strand DNA then will be loaded on a 12.5% polyacrilamide gel, 15°C for 2-5 hours, then stained with automated gel stainer and then sequenced.

Cell Culture and stable cell lines selection:

HEK293 cell were cultured in minimum essential medium contain 10% horse serum, at 37°C and 5% CO2. HEK293 cells were transfected with the respective plasmids using the calcium phosphate precipitation method. After 3 weeks of G418 selection, single colonies were screened by uptake transport study and immunoblot analysis.

Uptake transport assays:

HEK293 stable cell lines (wild type or mutation form) were seeded in 6-well dish, after 72 hours, cells were washed with transport medium (optiMEM; invitrogen) and treated with radiolabeled drug in the presence or absence of selection drug. At the various time intervals, cells were washed with ice-cold PBS then lysed with 1% SDS. Retained cellular radioactivity was quantified by liquid scintillation spectrometry.

Plasmid construction:

The eukaryotic expression construct for pcDNA3-OATPC, was kindly gifted by Jun-ichi Nezu (CHUGAI PHARMACEUTICAL CO., LTD).

Site directed mutagenesis:

Mutant (A388G) were constructed using the QuickChange Site-Directed Mutagenesis Kit (Stratagene) following the procedure recommended by the manufacturer. PCR primers for
introduction of mutations were:
5’ GTATTCTAAAGAAACTAATATCGATTCATCAGAAAATTC 3’
5’ GAATTTTCTGATGAAAAATTAC 3’

Second Year

To evaluate the pharmacokinetics of ticlopidine in Chinese with various OATP-C genotype.

From the 1st year results, we looked for the health volunteers (10 person in each genotype) for this study. After having approval from IRB of National Cheng Kung University Hospital and obtaining the consent form, all drugs were held at least for 1 week from these volunteers.

After overnight fasting and bladder emptied, each volunteer received a single oral dose of Licodin ® (Ticlopidine) 250 mg tablet with 150 ml water. Serial blood sampling was done through an indwelling catheter immediately before and 15 min, 30 min, 45 min, 1h, 2h, 3h, 4h, 6h, 8h, 12hr, 24hr after dosing. Predosing urine collection and 24 hour collect of urine were requested. After centrifuge, all samples were stored at -70℃ until quantitative analysis.

chromatographic conditions

The HPLC was consisted of two pumps a computerized integrator (Data System Model 450MT 2 from Kontron. Zurich, Switzer-land) and the analytical column (Supelcosil LC-8-DB, 15 cm*4.6 mm I.D., 5μm particle size; Supelco, Bellefonte, PA, USA) equipped with a guard column (Supelguard LC-8-DB, 2cm*4.6mm I.D., 5μm particle size; Supelco)

Analysis was carried out isocratically , using a mobile phase of acetonitrile-methanol-0.05MKH2PO3.0 containing 0.2% of triethylamine (20:25:55, v/v) filtered through a 0.45μm membrane (type HATV , Millipore). The flow-rate was 1.0 m/1 min and the effluent was monitored at 235 nm. Under these conditions, the retention times for ticlopidine and the I.S. were 7.6 and 11.6 min, respectively. (L. Dal Bo et al 1995)

The area under time-concentration curve (AUC) was calculated with trapezoid method and other pharmacokinetic profiles obtained from conventional methods. All the AUC and kinetic parameters in different genotypes were evaluated with student- t- test with an alpha values of 0.05.

Drug interaction between ticlopidine and Ergoloid Mesylate.

Approval of IRB and informed consent was obtained first.

An interval of at least 2weeks was waited for making sure that the ticlopidine had been completely excreted. We looked for 10 volunteers in each genotype to participate this trial. Ergoloid mesylate (Hydergine®) 1mg tablet was provided to the volunteers. They would be requested to take Hydergine® three times a day (after meal), each time with 1 tablet. In addition to tea, coffee, and wine. All the grape-fruit juice, orange juice, and apple juice were abstinent from the volunteers. On the 8th day, on which the volunteer suppose to have steady-state condition of Hydergine®, the similar procedure of part I was performed again after overnight fasting with a tablet of Licobin® (250mg) be given. The AUC and other pharmacokinetic parameters of ticlopidine both before and after 1
week-duration of Hydergine®, were evaluated with paired-t-test. With an alpha value of 0.05.

**Drug interaction between ticlopidine and Ginko Biloba.**

After approval from IRB, informed consent was obtained.

Another interval of at least 2 weeks was chosen again, to make sure that both ticlopidine and Hydergine® had be excreted completely. Then Ginko Biloba (Cerenin®) was used with 1 tablet three times a day for a week which very likely to bring up the serum concentration of Cerenin® into a study-state condition. On the morning of the 8th day, after overnight fasting, a tablet of 250 mg Licodin® was given and the same procedure repeated once again. The pharmacokinetic parameters of ticlopidine was compared (study I and II b, both before and after study-state concentration-reaching dose of Cerenin®) with paired-t-test with an alpha value of 0.05.

**Data Analysis**

The pharmacokinetics was characterized, when appropriate, by peak plasma drug concentration ($C_{\text{max}}$), time to peak concentration ($t_{\text{max}}$), and the area under the plasma drug concentration-time profile (AUC) from 0 to 12 hours (AUC$_{0-12}$) and from 0 hours to infinity (AUC$_{0-\infty}$). The $C_{\text{max}}$ and $t_{\text{max}}$ values were obtained directly from the experimental data. AUC$_{0-12}$ was determined by use of the linear trapezoidal rule.

**IV. Results & Discussion**

The results of SNP rate of OATP-C in normal Chinese people living at Taiwan are shown as Table 1. The genotypes and alleles distribution in Taiwanese and Japanese are shown as Table 2.

**Table 1.**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency(%) n=324</th>
<th>Genotype</th>
<th>Frequency(No.) n=162</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP-C*1a</td>
<td>22.22%</td>
<td>OATP-C*1a/1a</td>
<td>6 (3.70%)</td>
</tr>
<tr>
<td>OATP-C*1b</td>
<td>68.21%</td>
<td>OATP-C*1a/1b</td>
<td>51 (31.48%)</td>
</tr>
<tr>
<td>OATP-C*5</td>
<td>0 %</td>
<td>OATP-C*1b/1b</td>
<td>75 (46.30%)</td>
</tr>
<tr>
<td>OATP-C*15</td>
<td>9.57%</td>
<td>OATP-C*1a/15</td>
<td>9 (5.56%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OATP-C*1b/15</td>
<td>20 (12.35%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OATP-C*15/15</td>
<td>1 (0.62%)</td>
</tr>
</tbody>
</table>
Table 2.

<table>
<thead>
<tr>
<th>OATP-C genotype</th>
<th>Taiwan subjects@ (n =162)</th>
<th>Japanese subjects* (n = 120)</th>
<th>Japanese subjects# (n = 267)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1a / *1a</td>
<td>6 (3.7%)</td>
<td>13 (10.8%)</td>
<td>29 (10.9%)</td>
</tr>
<tr>
<td>*1a / *1b</td>
<td>51 (31.5%)</td>
<td>37 (30.8%)</td>
<td>115 (43.1%)</td>
</tr>
<tr>
<td>*1b / *1b</td>
<td>75 (46.3%)</td>
<td>26 (21.7%)</td>
<td>75 (28.1%)</td>
</tr>
<tr>
<td>*1a / *15</td>
<td>9 (5.6%)</td>
<td>14 (11.7%)</td>
<td>15 (5.6%)</td>
</tr>
<tr>
<td>*1b / *15</td>
<td>20 (12.4%)</td>
<td>17 (14.2%)</td>
<td>21 (7.9%)</td>
</tr>
<tr>
<td>*15 / *15</td>
<td>1 (0.6%)</td>
<td>1 (0.8%)</td>
<td>8 (3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>Taiwan subjects@ (n=324)</th>
<th>Japanese subjects* (n=240)</th>
<th>Japanese subjects# (n=534)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP-C*1a</td>
<td>72 (22.2%)</td>
<td>78 (32.5%)</td>
<td>188 (35.2%)</td>
</tr>
<tr>
<td>OATP-C*1b</td>
<td>221 (68.2%)</td>
<td>110 (45.8%)</td>
<td>287 (53.7%)</td>
</tr>
<tr>
<td>OATP-C*5</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>4 (0.7%)</td>
</tr>
<tr>
<td>OATP-C*15</td>
<td>31 (9.6%)</td>
<td>35 (15%)</td>
<td>55 (10.3%)</td>
</tr>
</tbody>
</table>

@ Data from this study.
* Data from Nishizato et al.
# Data from Nozawa et al.

The results of studies in effect of ergoloid mesylate and gingko biloba on ticlopidine pharmacokinetics are summarized as below. The mean plasma concentration of ticlopidine versus time and the summary pharmacokinetic parameters for ticlopidine are presented in Figure 1 and Table 3, respectively. Ergoloid mesylates administered with ticlopidine decreased plasma ticlopidine concentration compared to ticlopidine given alone (control), whereas coadministered ginkgo biloba did not affect plasma ticlopidine concentrations. When ergoloid mesylates was coadministered, the mean AUC$_{0-12}$ of ticlopidine was $1216.7 \pm 315.6$ ng.h/mL, and the mean $C_{max}$ was $516.2 \pm 165.1$ ng.h/mL, compared with means of $1732.1 \pm 329.6$ ng.h/mL and $723.6 \pm 184.0$ ng.h/mL for these parameters for controls. Coadministraion of ergoloid mesylates decreased the mean ticlopidine AUC$_{0-12}$ 30% and the mean $C_{max}$ 29% compared to control values. The mean AUC$_{0-12}$ and $C_{max}$ of ticlopidine were $1736.6 \pm 612.6$ ng.h/mL and $710.1 \pm 247.2$ ng.h/mL, when ginkgo biloba was coadministered. These values were not significantly different form control ticlopidine AUC$_{0-12}$ and $C_{max}$ values. On the other hand, no significant difference in ticlopidine $t_{max}$ was observed between ergoloid mesylates and control treatments.

The $t_{max}$ of ticlopidine was increased when ginkgo biloba was coadministered, but the increase was not significant.
Figure 1. Mean plasma concentration-time profiles of ticlopidine with and without ginkgo biloba (A) or ergoloid mesylates (B) in healthy volunteers.
Table 3.

Pharmacokinetic Parameters of Ticlopidine with and Without Ginkgo Biloba or Ergoloid Mesylates in Healthy Volunteers

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Ticlopidine (control)</th>
<th>Ticlopidine + Ginkgo Biloba</th>
<th>Ticlopidine + Ergoloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC 0-12, ng.h/mL</td>
<td>1732.1±329.6</td>
<td>1736.6±612.6</td>
<td>1216.7±315.6*</td>
</tr>
<tr>
<td>% of control</td>
<td>100</td>
<td>100.26</td>
<td>70.25</td>
</tr>
<tr>
<td>AUC0-∞, ng.h/mL</td>
<td>1767.7±320.0</td>
<td>1802.8±616.9</td>
<td>1229.5±317.2*</td>
</tr>
<tr>
<td>% of control</td>
<td>100</td>
<td>101.99</td>
<td>69.55</td>
</tr>
<tr>
<td>Cmax, ng.h/mL</td>
<td>723.6 ±184.0</td>
<td>710.1±247.2</td>
<td>516.2±165.1*</td>
</tr>
<tr>
<td>tmax, h</td>
<td>1.5</td>
<td>2</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*AUC 0-12,* area under plasma concentration-time curve from 0 to 12 hours; *AUC 0-∞,* area under plasma concentration-time curve from 0 to infinity; *Cmax,* peak concentration; *tmax,* time to peak concentration.

V. Reference


