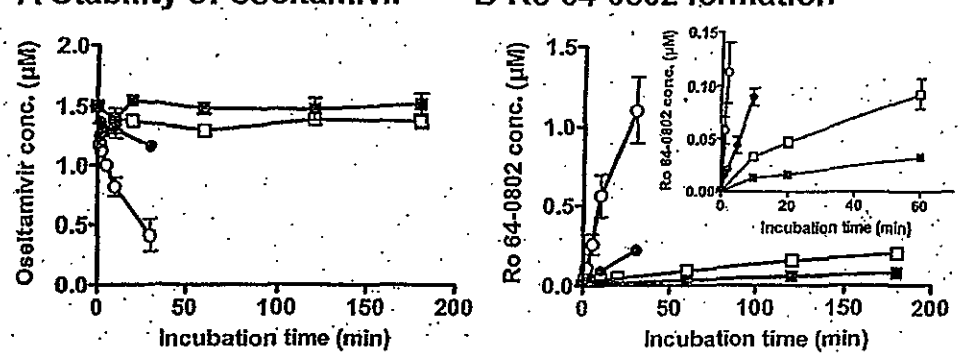

FIG. 7. The stability of oseltamivir (A) and the formation of Ro 64-0802 (B) in plasma and liver S9 from newborn and adult rats. Oseltamivir (1.5 μM) was incubated with rat plasma (circles) and liver S9 (squares) specimens (5 mg of protein/ml) for various time periods. The data for adult (P42) rats are shown by open symbols and for newborn (P11) rats by closed symbols. Data represent mean ± S.E. of three determinations using three plasma and liver S9 specimens prepared independently from three rats.

TABLE 1

Ro 64-0802 formation rate from oseltamivir in plasma and liver S9 from newborn and adult rats

Oseltamivir (1.5 μM) was incubated with rat plasma for 5 min and rat liver S9 fraction for 60 min at 37°C.

Source		Ro 64-0802 Formation Rate[a]		
		pmol/min/mg protein	*pmol/min/ml or g liver*[b]	*nmol/min/kg b.wt.*[c]
Plasma	P11	1.75 ± 0.32	87.7 ± 15.9	3.38 ± 0.61
	P42	10.6 ± 2.6	529 ± 129	20.4 ± 5.0*
Liver S9	P11	0.11 ± 0.00	10.6 ± 0.3	0.42 ± 0.01
	P42	0.31 ± 0.05	29.7 ± 4.5	1.19 ± 0.18#

* $P < 0.05$ statistical differences in Ro 64-0802 formation rate (nmol/min/kg) in plasma between newborn and adult rats.

\# $P < 0.05$ statistical differences in Ro 64-0802 formation rate (nmol/min/kg) in liver S9 between newborn and adult rats.

[a] Data represent means ± S.E. of three determinations using three plasma and liver S9 prepared independently from three rats.

[b] Obtained by multiplying the value (pmol/min/mg protein) by 50.0 and 96.1 for plasma and liver S9, respectively.

[c] Obtained by multiplying the value (pmol/min/ml plasma or g liver) by 38.5 and 40.0 for plasma and liver S9, respectively.

distribution volume of oseltamivir in the brain was greater than the capillary volume (Takasato et al., 1984; Rousselle et al., 1998), indicating that oseltamivir crosses the BBB. Pretreatment with GF120918, a dual inhibitor for P-gp and Bcrp (Allen et al., 1999), caused a significant increase in the brain concentration of oseltamivir. This is partly a result of greater plasma concentrations of oseltamivir in GF120918-treated group, presumably because of an inhibition of esterase activity by GF120918 as oseltamivir is predominantly converted to Ro 64-0802 in mice, and the biliary and urinary excretion account for a limited part of the systemic elimination, at most 0.3 and 19%, respectively (data not shown). However, a significant increase in the $K_{p,brain}$ of oseltamivir by GF120918 indicates that inhibition of active efflux mediated by P-gp and/or Bcrp is another underlying mechanism (Fig. 2B). Unlike oseltamivir, the distribution volume of Ro 64-0802 was close to the capillary volume, and $K_{p,brain}$ of Ro 64-0802 following oseltamivir or Ro 64-0802 administration was slightly increased by the administration of GF120918, but the difference was not statistically significant. This would be reasonable considering the low lipophilicity of Ro 64-0802 that will exhibit low BBB permeability without the aid of uptake transporters.

To support the effect of GF120918, in vivo studies using Mdr1a/1b and Bcrp knockout mice were performed. The $K_{p,brain}$ of oseltamivir was significantly increased in Mdr1a/1b P-gp knockout mice but not in Bcrp knockout mice (Fig. 3B). The increase in the $K_{p,brain}$ of Mdr1a/1b P-gp knockout mice was comparable with that obtained by GF120918 (Figs. 2B and 3B). Therefore, P-gp, but not Bcrp, limits the brain penetration of oseltamivir across the BBB. In accordance with the in vivo results, cellular accumulation study elucidated that both mouse Mdr1a P-gp and human P-gp accept oseltamivir as substrate because the cellular accumulation of oseltamivir was lower in a cell line expressing mouse P-gp and human P-gp, which was increased by PSC833 treatment (Fig. 8).

The present study elucidated that the activity of P-gp is an important factor for the brain concentration of oseltamivir in mice. Because abnormal behavior following oseltamivir medication is more frequently observed in younger generations than in adults, postnatal ontogeny of P-gp is an important issue. Mdr1a mRNA and P-gp protein levels were significantly lower in newborn rats than adult rats (Figs. 5 and 6). This result is in good agreement with previous reports, in which it has been shown that adults had higher brain expression of Mdr1a mRNA (3-fold) and a corresponding 5-fold increase in immunodetectable P-gp (Matsuoka et al., 1999; Goralski et al., 2006). Consistent with this ontogenic profile, the brain accumulation of cyclosporin A was 80% lower in adult mice than in 1-day-old mice (Goralski et al., 2006). NDA documents reported that the $K_{p,brain}$ of oseltamivir, obtained by comparison of the area under the curve of the plasma and brain concentration time profiles, was dramatically greater in newborn rats than that in adult rats at very high doses of oseltamivir (1000 mg/kg, p.o.). The brain concentrations of oseltamivir in newborn rats were significantly higher than those in adult rats (Fig. 4B). This is partly because of greater plasma concentrations of oseltamivir in newborn rats than in adult rats (Fig. 4A); however, a significant increase in the $K_{p,brain}$ of oseltamivir in newborn rats suggests that the smaller efflux clearance across the BBB is part of the underlying mechanism. This is in good agreement with the postnatal ontogenic profile of P-gp (Matsuoka et al., 1999; Goralski et al., 2006).

Newborn rats exhibited greater plasma concentrations of oseltamivir, suggesting a smaller systemic elimination rate in newborn rats. This was confirmed by comparing the conversion activities [carboxylesterase (CES) activity] in the plasma and liver S9 specimens between newborn (P11) and adult (P42) rats. Compared with adult rats, the conversion activities (CES activity) were lower in both the plasma and the liver S9 specimens from newborn rats (Fig. 7; Table 1). Lower conversion activity of oseltamivir to Ro 64-0802, particularly in the plasma, will account for the delay in the systemic elimination of oseltamivir in newborn rats.

Recent clinical studies support that P-gp acts as a gatekeeper protein in human BBB (Sadeque et al., 2000; Sasongko et al., 2005). P-gp will also be one of the determinant factors for the brain concentrations of oseltamivir. Single nucleotide polymorphisms (SNPs) are the genetic factor for interindividual differences in drug response. A number of SNPs have been described in the human MDR1 gene (Fromm, 2002; Kim, 2002). Of these, linkage disequilibrium has been shown between SNPs in exons 26 (C3435T), 21 (G2677T), and 12 (C1236T), and the TTT haplotype correlates with low P-gp activity in

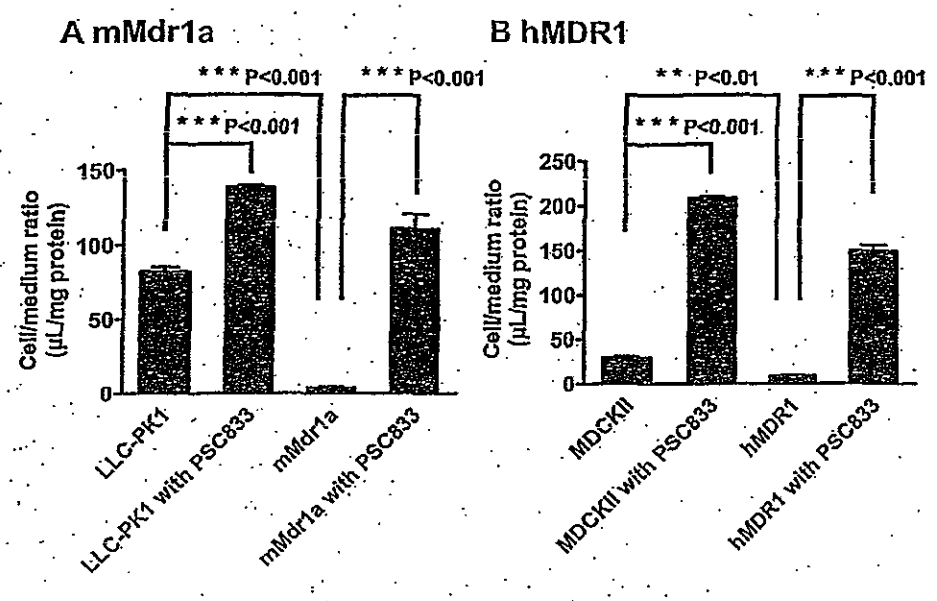

FIG. 8. Cellular accumulation of oseltamivir in; mMdr1a-LLC-PK1 cells (A) and hMDR1-MDCKII cells (B). The uptake of oseltamivir (2.5 μM) by mMdr1a-LLC-PK1 (A) and hMDR1-MDCKII (B) cells was examined in the presence or absence of PSC833 (5 μM) at 37°C. Each point represents the mean ± S.E. ($n = 4$). Statistical significance was calculated by one-way analysis of variance followed by Tukey's multiple comparison test.

the small intestine (Chowbay et al., 2003). As far as the BBB is concerned, there was no significant relationship between the haplotype and brain concentrations of [^{11}C]verapamil (Brunner et al., 2005; Takano et al., 2006). However, Kimchi-Sarfaty et al. (2007) recently reported that the effect of double or triple haplotypes containing C3435T on P-gp activity is "substrate dependent." The possibility that SNPs of P-gp are associated with an interindividual difference in the BBB permeability of oseltamivir cannot be excluded. In addition to P-gp, as observed in newborn rats, the activity of hCES1 is the determinant factor for the systemic elimination. C70F and R128H of hCES1 were reported to be associated with reduced hydrolysis of oseltamivir (Shi et al., 2006). Subjects with these SNPs of hCES1 will result in a greater exposure of oseltamivir to the brain.

Ro 64-0802 is a potent and selective inhibitor of influenza virus neuraminidase (sialidase). Several sialidases are expressed in the human brain and are suggested to be involved in the mitochondrial apoptotic pathway in neuronal cell death (Yamaguchi et al., 2005; Hasegawa et al., 2007). Inhibition of sialidases in the brain may be associated with the abnormal behavior following oseltamivir medication. Based on this speculation, production of Ro 64-0802 in the human brain will be the key event that triggers the central nervous system side effects. Unlike the ester-type prodrug, Ro 64-0802 barely penetrates into the brain from the circulating blood because of its hydrophilic property. As hCES1 is also expressed in the brain (Satoh et al., 2002), it is possible that Ro 64-0802 is formed in the brain from the oseltamivir. Because of low membrane permeability, Ro 64-0802, once produced in the brain from oseltamivir, may accumulate in the brain. It is also possible that Ro 64-0802 undergoes active efflux from the brain at the BBB because Ro 64-0802 is a substrate of renal OAT1 (*SLC22A6*) (Hill et al., 2002), and OAT3, the homolog of OAT1, is expressed at the BBB and actively eliminates organic anions from the brain (Kikuchi et al., 2003, 2004). This should be examined in the future.

In conclusion, the present study showed that oseltamivir crosses the BBB, but the active form Ro 64-0802 barely crosses the BBB. P-gp limits the brain penetration of oseltamivir at the BBB of adult mice. Ontogenetic profile of P-gp and CES activities accounts for the greater accumulation of oseltamivir in the brain of neonates at least in rats.

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FLASH

今月のFLASH

- タミフルが脳へ移行する可能性
- CO_2を吸収する地球の能力が低下
- クリック化学で細胞現象を追う
- ニンニクで高血圧に対抗できる理由
- タンパク質のリン酸化を光制御

タミフルが脳へ移行する可能性

オセルタミビル（商品名タミフル）は，インフルエンザの特効薬として知られているが，実際に体内で働いているのは活性体（Ro 64-0802）である（図1）．生体内でCES1という酵素により，加水分解され，活性体へと変換される．活性体はインフルエンザウイルスが感染細胞表面から遊離することを阻害して，ほかの細胞への感染・増殖を抑制する．タミフルは服用後の異常行動が注目され，社会問題化している．

タミフル服用と異常行動との因果関係については，これまで多くの議論がなされているが，最終的な結論には至っていない（本誌2006年12月号，p.8参照）．もし，異常行動がタミフル服用と関連があるとすると，タミフル（およびその活性体）が脳内へ入り，神経細胞に作用することを確認する必要がある．最近，血液から脳内へのタミフルの移行に関して，実験動物（マウス・ラット）を用いた解析結果が，高崎健康福祉大学の荻原琢男らのグループと，東京大学の杉山雄一らのグループからそれぞれ発表された（K. Morimotoほか，*Drug. Metab. Dispos.*, 36, 6(2008)；A. Oseほか，*Drug. Metab. Dispos.*, in press）．これらの内容を紹介したい．

タミフルの話に入る前に，血液と神経細胞の間にある“関門”のことを説明しよう（図2）．脳には，神経細胞に酸素や糖を供給するため，網目のように毛細血管が張り巡らされている．この毛細血管は血液脳関門ともよばれ，栄養素の供給だけではなく，神経細胞へ作用する医薬品を含めた多様な化学物質の脳への進入を制限するための関門としても働いている．

この関門は図2に示したように，単層の内皮細胞で構成されており，細胞間は発達した密着結合で塞がれている．必然的に，血液中から神経細胞側へと到達するためには，内皮細胞の細胞膜を通り抜ける必要がある．

神経細胞の機能維持に必要なアミノ酸や糖など，極性の高い栄養素に対しては，細胞膜透過を促進するような輸送メカニズム（トランスポーター）が備わっている（図2の①）．そうしたものを除けば，極性の高い分子は，血液脳関門を通り抜けることができない．脂溶性の高い化合物は内皮細胞を十分通り抜けることができる（図2の②）．

こうした化合物の透過を制限するために，P糖タンパク質が細胞膜上（血液側の細胞膜）で医薬品を内皮細胞内から血液側方向へと積極的に汲み出すことで，医薬品が脳に入ることを制限している（図2の③）．つまり，血液脳関門の関門機構は，密着結合に代表される静的な障

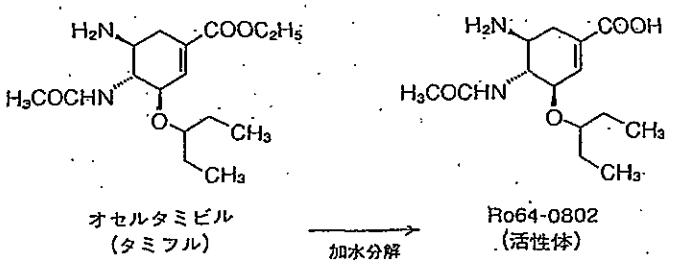

図1　タミフルの構造

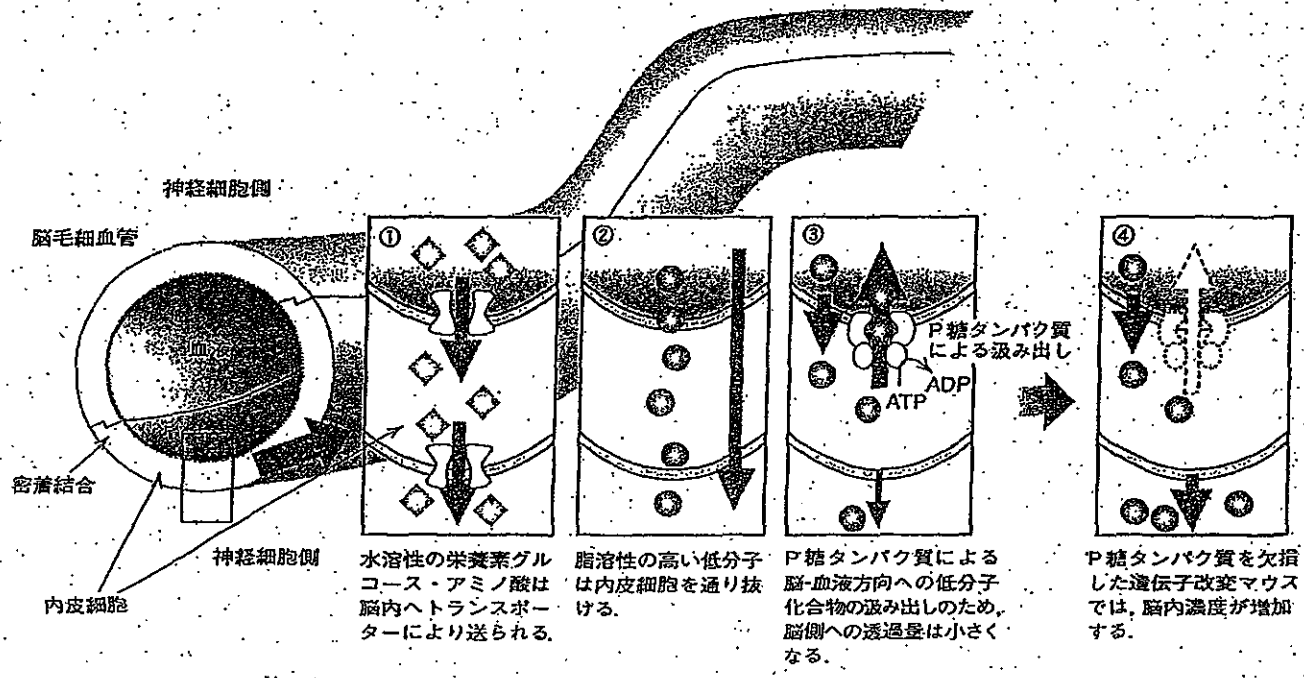

図2　脳毛細血管における物質透過

壁と，P糖タンパク質に代表される異物排除システムによる動的な障壁により構成されている．

タミフルに話を戻そう．上述の血液脳関門のメカニズムを考えると，水溶性の高い化合物を除いて，まったく脳内に入らないということはありえない．実際，マウスにタミフルを投与すると，脳内のタミフル量は毛細血管に残存している量をはるかに超えており，神経細胞周辺に移行していることがわかる．一方で，活性体自体の薬物量は毛細血管に残存している量と同程度であり，ほとんど血液脳関門を透過していないといえる．活性体はタミフルよりも極性が高いことから，合理的な結果といえる．

P糖タンパク質を過剰発現した細胞を用いた*in vitro*（生体外の）輸送試験の結果からは，タミフルがP糖タンパク質の基質となることが明らかにされた．P糖タンパク質が発現しないように遺伝子を改変したマウスにタミフルを投与すると，非改変マウス（野生型マウス）に比べて脳内のタミフル濃度が7倍近く高くなった．一方で，遺伝子改変マウスと非改変マウスとでは，活性体の脳内濃度は変わらなかった．つまり，タミフルは脳毛細血管でP糖タンパク質により汲み出され，脳内への移行は，ある程度制限されているといえる．さらに，生後2週間までの新生児ラットでは，脳毛細血管内皮細胞でのP糖タンパク質の発現量が低い上に，血液中からの消失が遅いこともあり，脳内のタミフル濃度は成熟ラットの6倍にもなる．

ヒト脳毛細血管も実験動物同様に関門として機能しており，P糖タンパク質が医薬品を血液中へと汲み出しに働いていることも報告されている（L. Sasongkoほか，*Clin. Pharmacol. Ther.*, 77, 503 (2005)）．タミフル服用後の血液中には，活性体に加えてタミフルも検出されていることから，タミフルはP糖タンパク質での汲み出しを受けるものの，少なくとも投与されたタミフルの一部は脳に移行していると考えられる．活性体については，血液脳関門透過性が低いことから，脳内でタミフルから生成されないかぎり，脳内濃度はかなり低いことが予想される．

タミフルの全合成を開発した東京大学の柴崎正勝教授が，タミフルのPET（陽電子放射断層撮影法）プローブ化を報告している（M. Moritaほか，*Bioorg. Med. Chem. Lett.*, in press）．放射線医学総合研究所でも，タミフルのPETプローブ化に成功しており，PETによりラット脳内におけるタミフルおよびその活性体の時間変化を測定することに成功している（平成19年12月11日のプレス発表）．可視化技術を用いてタミフルおよびその活性体が脳内にどの程度入るのか，ヒトで実証研究を行うことが可能になった．今後の臨床試験が期待される．

タミフルが脳内に入るという事実は，必ずしも異常行動とタミフル服用の因果関係を説明するものではない．有害作用も含めて，医薬品の効果は濃度に依存しており，仮に脳内に医薬品が入ったとしても，その濃度が十分に小さければ，作用の発現には至らない．タミフル（あるいはその活性体）が脳内で作用するタンパク質を解明し，臨床投与量で脳内に到達するタミフル濃度が，そうしたタンパク質に十分作用する濃度であるのか，あるいは異常行動がみられた患者では，脳内のタミフル濃度を増加させる（神経細胞への曝露を増やす）要因となる血液脳関門の機能破綻や，関連酵素（CES1）の活性低下などが見られるのかなど，まだまだ多くの裏づけ試験が必要である．

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Low Penetration of Oseltamivir and Its Carboxylate into Cerebrospinal Fluid in Healthy Japanese and Caucasian Volunteers▽†

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Oseltamivir is a potent, well-tolerated antiviral for the treatment and prophylaxis of influenza. Although no relationship with treatment could be demonstrated, recent reports of abnormal behavior in young individuals with influenza who were receiving oseltamivir have generated renewed interest in the central nervous system (CNS) tolerability of oseltamivir. This single-center, open-label study explored the pharmacokinetics of oseltamivir and oseltamivir carboxylate (OC) in the plasma and cerebrospinal fluid (CSF) of healthy adult volunteers over a 24-hour interval to determine the CNS penetration of both these compounds. Four Japanese and four Caucasian males were enrolled in the study. Oseltamivir and OC concentrations in CSF were low (mean of observed maximum concentrations [C_{max}], 2.4 ng/ml [oseltamivir] and 19.0 ng/ml [OC]) versus those in plasma (mean C_{max}, 115 ng/ml [oseltamivir] and 544 ng/ml [OC]), with corresponding C_{max} CSF/plasma ratios of 2.1% (oseltamivir) and 3.5% (OC). Overall exposure to oseltamivir and OC in CSF was also comparatively low versus that in plasma (mean area under the concentration-time curve CSF/plasma ratio, 2.4% [oseltamivir] and 2.9% [OC]). No gross differences in the pharmacokinetics of oseltamivir or OC were observed between the Japanese and Caucasian subjects. Oseltamivir was well tolerated. This demonstrates that the CNS penetration of oseltamivir and OC is low in Japanese and Caucasian adults. Emerging data support the idea that oseltamivir and OC have limited potential to induce or exacerbate CNS adverse events in individuals with influenza. A disease- rather than drug-related effect appears likely.

Oseltamivir is an orally administered anti-influenza agent of the neuraminidase inhibitor class. The ethyl ester prodrug oseltamivir is delivered orally as a phosphate salt and converted by hepatic esterases to the active metabolite oseltamivir carboxylate (OC) (10). OC specifically binds and inhibits the influenza virus neuraminidase enzyme that is essential for viral replication (21). In this way, oseltamivir limits the spread of influenza virus subtypes A and B within the infected host. When used as treatment, oseltamivir reduces the severity and duration of symptoms (22, 33), while prophylactic administration prevents their onset (9, 26).

In recent years, abnormal or delirious behaviors have been reported with a low incidence in young individuals with influenza who were also receiving oseltamivir (32). Cases arose most commonly in Japan but were also observed in Taiwan, Hong Kong, North America, Europe, and Australia. No causative association could be demonstrated, and similar events were also reported in the absence of oseltamivir (6, 12, 17, 24). Nevertheless, health and regulatory authorities in Japan and elsewhere have amended the product label to include precautions on the use of oseltamivir in young persons. These actions, and the associated media coverage, have fostered renewed interest in the central nervous system (CNS) tolerability of oseltamivir.

The currently available preclinical and clinical evidence suggests a low potential for oseltamivir to adversely affect CNS function, and no plausible mechanism for oseltamivir to cause CNS toxicity has been identified (32). However, only very limited data exist to describe the CNS penetration of oseltamivir and OC in humans. Equally little is known about the impact of ethnicity on the CNS profile of these entities. In this study, we investigated the pharmacokinetics of oseltamivir and OC in plasma and cerebrospinal fluid (CSF)—the latter being a recognized surrogate for CNS penetration (29)—in healthy adult volunteers after a single oral administration of oseltamivir phosphate. Although not powered to formally examine the impact of ethnicity on CNS penetration, we also considered whether any gross differences might exist by including both Caucasian and Japanese subjects in our study.

MATERIALS AND METHODS

Study design and subjects. This exploratory trial was a single-center, open-label, single-dose, pharmacokinetic study that was conducted in the United States between 16 July 2007 and 17 August 2007. The trial complied with the principles of the Declaration of Helsinki (as amended in Tokyo, Venice, Hong Kong, and South Africa). The study protocol and materials were approved by an independent ethics committee, and written informed consent was provided by all participants. The study fully adhered to good clinical practice guidelines (ICH Tripartite Guideline, January 1997).

The study aimed to recruit eight healthy adult male and female volunteers: four of Japanese origin (born in Japan of Japanese parents and grandparents and living for <5 years outside Japan) and four of Caucasian origin (white Hispanic or non-Hispanic). The numbers of males and females were intended to be the same in both ethnic groups. Inclusion criteria were age of 20 to 45 years, body mass index of 18 to 30 kg/m^2, and ability to give written informed consent and comply with the study restrictions. Female subjects were required to be surgically sterile or postmenopausal for ≥1 year or to use two methods of contraception (including one barrier method) from study commencement until 7 days postdosing. Exclusion criteria included clinically significant disease; allergy or immuno-

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