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Communication

Potential relationships between transaminase abnormality and valproic acid clearance or serum carnitine concentrations in Japanese epileptic patients

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Abstract

This study tested the hypothesis that the determinants of mild liver injury are prerequisites for more severe idiosyncratic hepatotoxicity. This study verified whether the possible risk factors for rare idiosyncratic valproic acid (VPA)-induced hepatotoxicity, VPA clearance and/or serum carnitine concentrations are common to those for a mild elevation in transaminases in VPA-treated patients. VPA clearance was calculated in 172 Japanese patients with epilepsy, using a non-linear mixed-effects regression program. Carnitine concentrations were determined in a subset of 60 patients. The relationships between VPA clearance, carnitine concentration and levels of transaminases and ammonia were evaluated by Pearson's correlation coefficients. The final model of VPA apparent clearance (CL/F) was as follows: $CL/F\ (L\ h^{-1}) = 0.012 \times (BW/40)^{0.34} \times dose^{0.55} \times 0.90^{gender} \times 1.32^{PHT} \times 1.11^{CBZ} \times 1.12^{PB}$, where BW = total body weight (kg); gender = 1 if female, 0 if male; PHT/CBZ/PB = 1 if phenytoin, carbamazepine, or phenobarbital, respectively, is coadministrated, otherwise 0. Either a higher VPA clearance or acyl/free carnitine ratio and a lower total and/or free carnitine concentration, but not VPA concentration, were associated with the mild elevation in transaminases or ammonia. These results support the initial hypothesis, while also helping to clarify the mechanism of severe idiosyncratic hepatotoxicity with VPA.

Introduction

Valproic acid (VPA) is well established as a first-line antiepileptic agent, with a broad spectrum of activity against both generalized and partial seizures (Perucca 2002). More recently, the range of indications for VPA has increased and now includes bipolar disorder and other psychiatric disorders such as alcohol withdrawal and dependence, reduction of cocaine use, agitation associated with late-life psychosis, and Alzheimer's disease (Denicoff et al 1997; Peterson & Naunton 2005). Moreover, VPA has been found to exert an anti-tumour effect, acting as a histone deacetylase inhibitor (Peterson & Naunton 2005; Atmaca et al 2007).

While VPA is usually well tolerated, idiosyncratic hepatotoxicity is a cause for concern (Kreher et al 2002; Koenig et al 2006). Two types of VPA-associated hepatotoxicity have been distinguished (De Vivo et al 1998; Lheureux et al 2005; Koenig et al 2006). Type I consists of a dose-dependent elevation of serum liver enzymes, which normalize after discontinuation of VPA or with carnitine supplementation. Type II VPA-associated hepatotoxicity is a rare, irreversible idiosyncratic reaction that is usually lethal. From a clinical point of view, it is therefore important to determine whether there is a clinical sign or marker that can predict hepatotoxicity of VPA. A reduction in the dose of VPA on the basis of such a sign or marker may lead to an improvement in the clinical and laboratory parameters without requiring discontinuation of therapy.

The established risk factors for VPA-induced hepatotoxicity are age under 2 years and polytherapy (VPA with enzyme-inducing anti-epileptic drugs such as phenytoin, carbamazepine and phenobarbital), and these are also risk factors for carnitine deficiency (Bryant & Dreifuss 1996; De Vivo et al 1998; Perucca 2002; Lheureux et al 2005). In contrast to previous concepts, however, Koenig et al (2006) reported that in Germany from 1994 to 2003, all patients with fatal hepatotoxicity and 87% of patients with reversible hepatotoxicity were older than 5 years. They therefore warned that the risk of VPA-induced hepatotoxicity is not limited to patients younger than 2 years who are receiving polytherapy, and patients with congenital or acquired metabolic diseases. It has been suggested that susceptibility to VPA hepatotoxicity could be enhanced by disturbing VPA metabolism and by causing secondary carnitine deficiency (Kondo et al 1992). Similarly, in a recent review of idiosyncratic drug hepatotoxicity, Kaplowitz (2005) proposed a hypothesis that the determinants of mild injury, such as disturbance of hepatic drug handling or intracellular detoxification, may be prerequisites for more severe idiosyncratic hepatotoxicity, while the latter requires the contribution of one or more additional rare determinants. The principal pathways of VPA metabolism are glucuronidation and β-oxidation, but VPA is also metabolized by cytochrome P450 (CYP) enzymes, especially when the enzyme-inducing antiepileptic drugs are co-administered (Levy et al 1990; Kondo et al 1992; Kreher et al 2002). Susceptibility to hepatotoxicity is considered to be associated with genetic polymorphisms in the genes encoding these CYP enzymes. Individual susceptibility to idiosyncratic hepatotoxicity is determined by the interaction of metabolic and immunological factors (Kaplowitz 2005; Watkins & Seeff 2006). A constitutional deficiency in another cell defence mechanism, which remains to be characterized, seems to significantly increase the risk of hepatotoxicity with phenytoin, carbamazepine and phenobarbital, and it is possible that a special mechanism exists for VPA hepatotoxicity.

VPA therapy is associated with metabolic disorders, including a state of secondary carnitine deficiency, hyperinsulinaemia, dyslipidaemia and hyperandrogenism (De Vivo et al 1998; Raskind & El-Chaar 2000; Perucca 2002; Foster 2004; Lheureux et al 2005; Pylvänen et al 2006). Carnitine is an essential amino acid, necessary for the β-oxidation of fatty acids and energy production in cellular mitochondria (Foster 2004). The role of carnitine as an adjunct to VPA therapy is to restore β-oxidation to mitochondrial cells of the liver and accept toxic acyl moieties from CoA. The efficacy of L-carnitine treatment for VPA hepatotoxicity has been confirmed by Bohan et al (2001), who reported that all of the patients recovered from idiosyncratic hepatotoxicity after discontinuation of VPA together with L-carnitine therapy (Koenig et al 2006). In addition, the administration of carnitine is safe (LoVecchio et al 2005). However, the beneficial effect of L-carnitine supplementation in preventing VPA-induced hepatotoxicity is unknown.

This study investigated whether the potential risk factors for VPA-induced idiosyncratic hepatic failure – a disturbance of VPA metabolism and carnitine deficiency – could be common to the mild elevation in transaminases among Japanese patients treated with VPA.

Methods

A total of 172 Japanese patients with epilepsy who had no history of either viral or alcoholic liver diseases and had undergone therapeutic drug monitoring of VPA at Kumamoto Saishunso National Hospital after January 1996 were assigned to the pharmacokinetic study population. Total carnitine (TC), free carnitine (FC) and acylcarnitine concentrations in the blood were determined in 60 subjects who visited the hospital from April 1 2002 to January 31 2005, using a previously described method (Takahashi et al 1994). All patients and/or their parents gave written informed consent to participate in the study. The protocol was approved by the ethics committees of Kumamoto Saishunso National Hospital and the Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University.

VPA pharmacokinetics was analysed using a non-linear mixed-effects regression program, WinNonMix (version 2.0.1; Pharsight, Mountain View, CA, USA). A one-compartment model was used, and a regression model was developed by use of the forward-inclusion and backward-elimination methods. The covariates evaluated were age, gender, total body weight (BW), VPA daily dose and coadministration of carbamazepine, phenytoin, phenobarbital, zonisamide or clonazepam. Each covariate was incorporated non-linearly in a stepwise manner into the basic regression model. The full model was created by incorporating all covariates, which thus led to the identification of a significant relationship. The influence of the fixed effects was evaluated by removing each covariate from the full model. The difference in the objective function values, assumed to be asymptotically chi-squared distributed, was used to assess statistical significance of the fixed effects during the forward-inclusion and backward-elimination analysis (likelihood ratio test); a difference in the objective function of at least 3.8 (chi-squared, $P < 0.05$, $\mathrm{df} = 1$) was considered to be significant.

We investigated the relationship between VPA clearance (CL) obtained from the final model and the carnitine concentrations and acyl/free carnitine ratio (A/F ratio), and evaluated their effects on the blood levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP) and ammonia by Pearson's correlation test. Differences with a P value below 0.05 were considered to be significant. Statistical analyses were performed using the SPSS software package (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics of the patients are shown in Table 1. Five subjects fulfilled the criteria of carnitine deficiency (i.e. FC concentration $\leq 20\,\mu\mathrm{mol\ L^{-1}}$ or A/F ratio $\geq$ 0.4 (De Vivo et al 1998), four of whom had elevated levels of transaminase(s) and/or ammonia. Since age and body weight (BW) correlated closely in the forward-inclusion analysis, the BW was incorporated in the mean apparent oral clearance (CL/F) model. Discrete covariates were examined according to a multiplicative model with a forward stepwise

Table 1 Summary of patient data, given as mean ± s.d. (range)

Demographic data	Pharmacokinetic study	Statistical analysis
Number of patients	172	60
Men/Women	100/72	32/28
Age (years)	15.8 ± 9.2 (0.4–46.8)	15.8 ± 7.5 (3.1–45.0)
Body weight (kg)	38.6 ± 16.2 (7.0–75.0)	46.3 ± 18.6 (12.0–99.0)
VPA dose (mg per day)	861 ± 517 (150–2700)	812 ± 489 (200–2700)
VPA concn (μg mL^{-1})	72.6 ± 24.9 (30.1–165.0)	68.9 ± 25.4 (29.4–144.5)
TC (μmol L^{-1})	–	46.0 ± 12.0 (20.2–66.0)
FC (μmol L^{-1})	–	37.2 ± 10.9 (14.6–57.0)
AC (μmol L^{-1})	–	8.9 ± 2.5 (5.0–16.5)
A/F ratio	–	0.26 ± 0.09 (0.12–0.54)
VPA CL (L h^{-1})	–	0.49 ± 0.22 (0.17–1.14)
AST[a] (IU L^{-1})	–	24.0 ± 9.1 (13.0–52.0)
ALT[a] (IU L^{-1})	–	19.8 ± 13.9 (6.0–80.0)
γ-GTP[a] (IU L^{-1})	–	64.2 ± 109.4 (9.0–623.0)
Ammonia[a] (μg dL^{-1})	–	66.2 ± 41.0 (22.0–232.0)
Number of observations	530	60
Coadministration		
VPA monotherapy	232	25
Carbamazepine	121	17
Phenytoin	47	5
Phenobarbital	60	4
Zonisamide	50	3
Clonazepam	51	1
F72.1 or F73.1	31	6

[a]Upper limits of normal: AST 31 IU L^{-1}; ALT 34 IU L^{-1}; γ-GTP 45 IU L^{-1} for men, 35 IU L^{-1} for women; ammonia 80 μg dL^{-1}

BW, total body weight; VPA, valproic acid; TC, total carnitine concentration; FC, free carnitine concentration; AC, acylcarnitine concentration; A/F ratio, acyl/free carnitine concentration ratio; VPA CL, estimated VPA clearance; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase; F72.1 or F73.1, severe or profound mental retardation with significant behaviour impairment, according to the ICD-10 criteria.

method. Gender and the coadministration of phenytoin, carbamazepine or phenobarbital showed a significant relationship with VPA CL/F. Table 2 shows the result of a backward-elimination step in the full model adjusted for each significant covariate. The final model of the CL/F was as follows: $\text{CL/F (L h}^{-1}) = 0.012 \times (\text{BW}/40)^{0.34} \times \text{dose}^{0.55} \times 0.90^{\text{gender}} \times 1.32^{\text{PHT}} \times 1.11^{\text{CBZ}} \times 1.12^{\text{PB}}$, where BW = total body weight (kg); gender = 1 if female, 0 if male; PHT/CBZ/PB = 1 if phenytoin, carbamazepine, or phenobarbital, respectively, is coadministered, otherwise 0.

The coefficients of variation of the estimated interpatient variability and residual intrapatient variability in the population CL of VPA were 19.9% and 9.9%, respectively.

The VPA CL correlated weakly with the TC and FC concentrations and the A/F ratio (Table 3). The VPA CL was also associated with the levels of ALT and γ-GTP. The TC and FC concentrations were associated with the levels of γ-GTP and ammonia. The A/F ratio was associated with concentrations of AST, ALT and γ-GTP (Table 3). Although the VPA daily dose and VPA daily dose/BW showed the same mode of correlation as the VPA CL, their correlations were much weaker than those of the VPA CL. Moreover, the VPA concentration was not associated with any markers.

Table 2 Summary of the NON-MEM analysis by backward-elimination method and final parameter estimates

Covariates	DOBF	*P* value	Conclusion
Body weight (kg)	−94.5	< 0.001	Yes
Daily dose of valproic acid (mg)	−338.8	< 0.001	Yes
Gender	−13.3	< 0.001	Yes
Coadministration			
Phenytoin	−40.2	< 0.001	Yes
Carbamazepine	−13.1	< 0.001	Yes
Phenobarbital	−9.9	< 0.005	Yes

DOBF, difference in objective function.

Discussion

In this study, higher VPA CL or A/F ratio and lower TC or FC concentration, but not the VPA concentration, were associated with mild elevations in transaminases or ammonia in Japanese patients with epilepsy treated with VPA. To our knowledge, this is the first report to document a significant

Table 3 Correlation coefficient between VPA CL, dose, D/B, carnitine concentrations and hepatic functions; Pearson's correlation coefficients are given in the right upper part; *P* values are given in the left lower part

	VPA CL	Dose	D/B	Concn	TC	FC	AC	A/F	AST	ALT	γ-GTP	Ammonia
VPA CL		0.93	0.57	0.31	−0.32	−0.37	0.03	0.39	0.19	0.40	0.49	0.28
Dose	< 0.001		0.75	0.54	−0.35	−0.40	0.05	0.40	0.23	0.31	0.36	0.15
D/B	< 0.001	< 0.001		0.56	−0.41	−0.46	0.03	0.44	0.16	0.17	0.27	0.21
Concn	0.046	< 0.001	< 0.001		−0.22	−0.26	0.12	0.22	−0.01	−0.09	−0.01	0.39
TC	0.012	0.007	0.001	0.161		0.98	0.53	−0.53	−0.05	−0.17	−0.44	−0.42
FC	0.004	0.002	< 0.001	0.093	< 0.001		0.35	−0.68	−0.11	−0.21	−0.48	−0.42
AC	0.816	0.689	0.833	0.441	< 0.001	0.006		0.38	0.25	0.12	−0.06	−0.18
A/F	0.002	0.001	< 0.001	0.152	< 0.001	< 0.001	0.003		0.33	0.41	0.56	0.27
AST	0.211	0.121	0.284	0.954	0.728	0.451	0.097	0.027		0.80	0.44	0.14
ALT	0.006	0.034	0.249	0.586	0.259	0.156	0.433	0.005	< 0.001		0.72	0.24
γ-GTP	< 0.001	0.017	0.075	0.946	0.002	0.001	0.707	< 0.001	0.002	< 0.001		0.31
Ammonia	0.155	0.441	0.282	0.050	0.027	0.024	0.357	0.167	0.473	0.224	0.120	

VPA, valproic acid clearance; CL, clearance; D/B: VPA daily dose/body weight; TC, total carnitine concentration; FC, free carnitine concentration; AC, acylcarnitine concentration; A/F, acyl/free carnitine concentration ratio; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma glutamyl transpeptidase.

relationship between VPA CL and levels of transaminases, and between the TC or FC concentration and the level of γ-GTP. In our subjects, the A/F ratio and FC concentration correlated most closely with the elevation of transaminases and ammonia, respectively.

Raskind & El-Chaar (2000) have extensively reviewed the pathophysiology and significance of VPA-induced carnitine deficiency while also evaluating the literature pertaining to carnitine supplementation during VPA therapy in children. Despite the lack of prospective randomized clinical trials, a few studies have shown that carnitine supplementation in patients receiving VPA results in subjective and objective improvements, and thereby prevents VPA-induced hepatotoxicity, in parallel with increases in the carnitine serum levels (Raskind & El-Chaar 2000; Bohan et al 2001; Lheureux et al 2005). In 1996, the Pediatric Neurology Advisory Committee strongly recommended carnitine supplementation during VPA therapy for children at risk for developing a carnitine deficiency, after a VPA overdose and also for patients with VPA-induced hepatotoxicity (De Vivo et al 1998). However, several issues remain unsettled regarding the role of carnitine supplementation during VPA therapy, such as the correlation between serum and muscle carnitine concentrations (since carnitine is stored mainly in muscle). Furthermore, patients with normal serum carnitine concentrations have also been reported to develop VPA-induced hepatic failure (De Vivo et al 1998; Raskind & El-Chaar 2000; Lheureux et al 2005). It is worth noting that a mild increase in the A/F ratio within reference limits was clearly associated with increased levels of AST, ALT and γ-GTP in this study.

VPA CL increased by 32%, 11% and 12%, respectively when phenytoin, carbamazepine and phenobarbital were coadministered, all of which are inducers of CYPs and uridine diphosphate glucuronosyltransferase (Levy et al 1990). The induction of CYPs is not expected to alter VPA CL appreciably because these pathways account for < 10% of the dose (Levy et al 1990; Perucca 2002). The mechanism of hepatotoxicity is unknown, and different hypotheses have been proposed. One possible mechanism is that the mediator responsible for VPA-induced hepatotoxicity is produced by the CYP-dependent formation of reactive metabolites, such as 4-ene-VPA and its subsequent mitochondrial β-oxidation metabolite (E)-2,4-diene-VPA (Levy et al 1990; De Vivo et al 1998; Kreher et al 2002). The other involves the generation of reactive oxygen species upon VPA exposure, a phenomenon which may or may not be dependent on VPA biotransformation (Chang & Abbott 2006). Regardless of the responsible mediator, the present study showed that the VPA CL correlated more closely with levels of ALT and γ-GPT than the VPA dose (Table 3). While physicians may correlate enzyme levels with the VPA concentration in routine clinical practice, there was no correlation between the VPA concentration and transaminase or carnitine levels in our study (Table 3); levels in patients with high ($\geq 100\,\mu g\,mL^{-1}$) or low ($< 100\,\mu g\,mL^{-1}$) serum VPA concentrations were similar (data not shown).

The induction of transaminases as well as CYPs has been well documented for anticonvulsants (Amacher 1998). Elevated circulating levels of γ-GTP are commonly observed in alcohol drinkers and patients taking enzyme-inducing antiepileptic drugs without symptoms of hepatic dysfunction. Therefore, a mild elevation in transaminases was not considered to be clinically significant, while also suggesting that there is no value in the routine performance of liver function tests in patients with epilepsy (Wall et al 1992; Amacher 1998). However, recent large prospective studies demonstrated that slightly increased levels of serum γ-GTP or ALT within reference limits was associated with increasing risks of cardiovascular disease or insulin resistance, with a graded response relationship (Emdin et al 2005; Ruttmann et al 2005; Burgert et al 2006; Lee et al 2006). Moreover, the clinical relevance of a mild elevation in transaminase levels due to the metabolic syndrome in VPA-treated patients has also been observed. Luef et al (2004) found that 61% of their patients treated with VPA had non-alcoholic fatty liver disease as another feature of the metabolic syndrome. Obese

patients treated with VPA tend to exhibit high serum insulin levels, thus indicating insulin resistance, and some of them have additional cardiovascular risk factors such as dyslipidaemia and endocrine disorders (Luef et al 2004; Pylvänen et al 2006). In addition, VPA has a particular effect on the microcirculation, elevating the density of capillaries and the tortuous index (Gerstner et al 2006). ALT is the enzyme most closely correlated with fat accumulation in the liver, and a recent study has suggested that elevations in ALT may not only be a marker of liver injury and a surrogate for fatty liver disease but also an early indicator of impending diabetes in children (Burgert et al 2006). ALT may therefore be a useful indicator of hepatic dysfunction and thus adverse metabolic effects of VPA therapy. On the other hand, the serum γ-GTP activity, which was found to be increased in 36% of patients in our study, has been reported to be independently associated with cardiovascular mortality (Emdin et al 2005; Ruttmann et al 2005; Lee et al 2006). The carnitine/carnitine palmitoyltransferase system is also involved in the pathogenesis of steatohepatitis, insulin resistance, the metabolic syndrome and cardiovascular disease, and the benefits of carnitine supplementation in patients with these conditions has also been suggested (Ferrari et al 2004; Foster 2004). While no data are available so far, L-carnitine supplementation may be expected to prove effective in preventing not only idiosyncratic hepatic dysfunction but also the diseases mentioned above after VPA treatment in adults; the risk of VPA-induced hepatotoxicity is not limited to children (Koenig et al 2006) and VPA has been reported to be associated with a higher risk of hypocarnitinaemia in adolescents and young adults (Coppola et al 2006).

It should be noted that the metabolism of VPA differs between Japanese and Caucasian patients with epilepsy. VPA CL is 2–3 times higher in Caucasians than in Japanese patients. VPA CL in a 40 kg boy (VPA daily dose 900 mg monotherapy) in the present study was 0.51 L h^{-1}; in another Japanese population VPA CL was 0.76 L h^{-1} (Yukawa et al 2003) and in a Caucasian population was 1.34 L h^{-1} (Serrano et al 1999). Thus, our results should be verified in other ethnic populations.

One of shortcoming of this retrospective study in routine clinical practice was that we did not observe any clinical signs of typical hepatic injury in the group demonstrating a positive correlation between VPA clearance and elevated levels of transferases. Also, the study design could not determine the independent effect of the enzyme-inducing antiepileptic drugs, with and without VPA, on the levels of transaminases. We therefore additionally compared the levels of transaminases between the subjects taking carbamazepine, phenytoin or phenobarbital with or without VPA. The levels of AST and ALT did not differ between the groups, but levels of γ-GTP were significantly higher in subjects also taking VPA than in those without (data not shown). In addition, despite the fact that the subjects investigated in this study represent a heterogeneous population, the number of subjects was too small to analyse the data in terms of age and BW, and there were no subgroups of patients with various comedications. These confounding factors could play an important role in the development of hepatic dysfunction, and were incorporated into the model of the CL/F.

Conclusion

Our results suggest that an increase in VPA CL or A/F ratio or reduction in TC and/or FC concentrations, even within reference limits, may be risk factors for a mild elevation of transaminase levels, supporting the hypothesis that the determinants of mild liver injury, such as a disturbance of hepatic drug handling or intracellular detoxification, may be prerequisites for more severe idiosyncratic hepatotoxicity. Further prospective long-term follow-up studies to evaluate whether VPA monotherapy alters liver functions, while also comparing subjects with and without these potential risk factors, are required to clarify the clinical significance of these findings.

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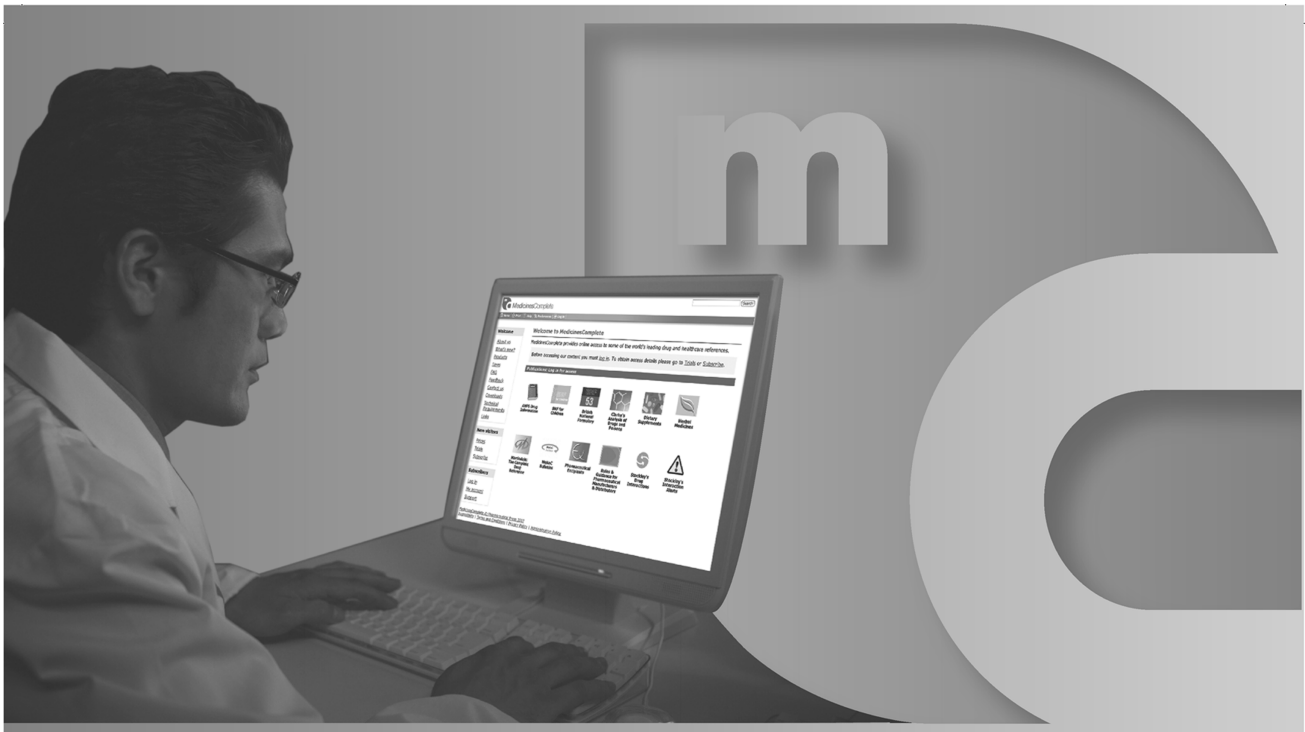

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