Pharmacokinetics and response to pravastatin in paediatric patients with familial hypercholesterolaemia and in paediatric cardiac transplant recipients in relation to polymorphisms of the *SLCO1B1* and *ABCB1* genes

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Aims

Our aim was to investigate associations between the single nucleotide polymorphisms (SNPs) in the *SLCO1B1* (encoding OATP1B1) and *ABCB1* (encoding P-glycoprotein) genes with the pharmacokinetics and efficacy of pravastatin in children with heterozygous familial hypercholesterolaemia (HeFH) and in paediatric cardiac transplant recipients.

Methods

Twenty children with HeFH (aged 4.9–15.6 years) and 12 cardiac transplant recipients (aged 4.4–18.7 years and receiving triple immunosuppressive medication) who had participated in previous pharmacokinetic and pharmacodynamic studies with pravastatin were genotyped for the –11187G > A and 521T > C SNPs in the *SLCO1B1* gene and for the 2677G > T/A and 3435C > T SNPs in the *ABCB1* gene.

Results

Two HeFH patients with the –11187GA genotype had a 81% lower peak plasma pravastatin concentration (C_{max}) (difference in means -13.9 ng ml^{-1} , 95% CI -21.1 , -6.7 ; $P < 0.001$) and a 74% smaller area under the plasma concentration-time curve ($AUC(0, \infty)$) ($-25.3 \text{ ng ml}^{-1} \text{ h}$, 95% CI -35.6 , -15.0 ; $P < 0.0001$) and significantly greater increase in high density lipoprotein (HDL) cholesterol after 2 months treatment with pravastatin than patients with the reference genotype. No significant differences were seen in the pharmacokinetics or effects of pravastatin between HeFH patients with the *SLCO1B1* 521TC and 521TT genotypes. The cardiac transplant recipients with the *SLCO1B1* 521TC genotype ($n = 3$) had a 46% lower C_{max} (-67.7 ng ml^{-1} , 95% CI -135.7 , 0.3 ; $P = 0.055$) and 62% lower $AUC(0,24 \text{ h})$ ($-228.5 \text{ ng ml}^{-1} \text{ h}$, 95% CI -402.7 , -54.3 ; $P = 0.016$) and a shorter half-life ($t_{1/2}$) (0.9 ± 0.1 vs. $1.3 \pm 0.4 \text{ h}$, $P = 0.015$) of pravastatin than those with the reference genotype. Decreases in total and low-density lipoprotein cholesterol by pravastatin were significantly smaller, and the increase in HDL-cholesterol was greater in the transplant recipients with the 521TC genotype compared with patients with the 521TT reference genotype.

Conclusions

In children with HeFH and in paediatric cardiac transplant recipients receiving immunosuppressive medication, the $-11187G > A$ and $SLCO1B1$ 521T > C SNPs were associated with decreased plasma concentrations of pravastatin. These differences are opposite to those seen previously in healthy adults. The mechanisms underlying these phenomena are unclear and warrant further study.

Introduction

The plasma concentrations and the cholesterol-lowering efficacy of pravastatin, a hydrophilic, semisynthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, vary considerably between individuals, both adults [1] and children [2]. However, no clear difference in the pharmacokinetics of pravastatin exists between hypercholesterolaemic and normocholesterolaemic adults [3–5]. Pravastatin is currently approved by the US Food and Drug Administration for the treatment of children over 8 years of age with familial hypercholesterolaemia (FH). In adult cardiac transplant recipients, pravastatin slows the development of accelerated coronary artery disease, and decreases the rates of cardiovascular morbidity and mortality [6]. Because progressive coronary artery disease is also the main cause of poor long-term survival in paediatric cardiac transplant recipients [7, 8], the tolerability, safety and efficacy of pravastatin in this patient group is currently being evaluated [9, 10].

The oral bioavailability of pravastatin is about 20% and it is cleared by both renal and non-renal routes (47% and 53%, respectively) [5, 11]. The metabolites of pravastatin include 3 α -iso-pravastatin and 6-epi-pravastatin, which are formed by nonenzymatic acid-catalyzed isomerization in the stomach or produced in the cytosol of liver and small intestinal mucosa cells, and 3 α , 5 β -dihydroxy-pravastatin and 3-hydroxypravastatin, which are the major oxidized metabolites [5]. Unlike many other statins, pravastatin is not significantly biotransformed by cytochrome P450 (CYP) enzymes [12], and thus it is not susceptible to CYP3A4- [1], CYP2C9- [13] or CYP2C19-mediated [13] drug interactions. Cyclosporin is a known inhibitor/substrate of CYP3A4 [14], P-glycoprotein [15], organic anion transporting polypeptide 1B1 (OATP1B1; previously known as OATP-C, OATP2 and LST-1) [16] and multidrug resistance-associated protein 2 (MRP2) [17], and it greatly elevates the plasma concentrations of many statins, including pravastatin [10, 18–20], and increases the risk of myopathy and rhabdomyolysis during statin therapy [21, 22].

OATP1B1 is responsible for the hepatic uptake of pravastatin [23, 24]. OATP2B1 (OATP-B) transports pravastatin from gut lumen into the cytosol of intestinal

epithelial cells and may facilitate its absorption [25]. MRP2 transports absorbed pravastatin back into the gut lumen and also mediates the biliary excretion of pravastatin from hepatocytes [26]. The organic anion transporter 3 (OAT3), a member of the *SLC22* superfamily, may affect the urinary excretion of pravastatin [27]. In adults, the 521T > C (Val174Ala) and $-11187G > A$ single nucleotide polymorphisms (SNPs) in the *SLCO1B1* gene encoding for OATP1B1 have been associated with increased plasma concentrations of pravastatin [28–30], whereas SNPs in *SLCO2B1* (encoding OATP2B1) [29], *SLC22A8* (encoding OAT3) [28], *ABCC2* (encoding MRP2) [29] or *ABCB1* (encoding P-glycoprotein) genes [29], have not affected its pharmacokinetics.

We have recently studied the pharmacokinetics and efficacy of pravastatin in paediatric patients with heterozygous familial hypercholesterolaemia (HeFH) who received no other concomitant medication [2] and in paediatric cardiac transplant recipients on a regimen of triple immunosuppression [10]. As the *SLCO1B1* 521T > C and $-11187G > A$ SNPs are associated with increased plasma pravastatin concentrations in adults, we investigated possible associations of these SNPs with the pharmacokinetics and efficacy of pravastatin in children. Moreover, as cyclosporin is a substrate of P-glycoprotein, we hypothesized that *ABCB1* SNPs might modulate the effects of cyclosporin on pravastatin. Thus, associations between the *ABCB1* 3435C > T and 2677G > T/A SNPs and pravastatin pharmacokinetics were also evaluated.

Methods

Patients

Twenty children (13 girls and seven boys) with HeFH, who had participated in our previous pravastatin study [2], were included. Their mean age was 10.3 ± 2.9 years and their other characteristics are listed in Table 1. The diagnosis of HeFH had been verified by LDL receptor mutation analysis [31] or by a lymphocyte test [32], as previously described [2]. Ten patients tested were positive for the FH-Helsinki LDL receptor mutation, six patients for the FH-North Karelia LDL receptor mutation, and four patients had defective cholesterol intake in the lymphocyte test. Other than HeFH, all patients

Table 1

Characteristics of 20 children with familial hypercholesterolaemia (HeFH) and 12 paediatric cardiac transplant recipients

	Sex	Age (years)	Weight (kg)	Height (cm)	Pravastatin dose (mg kg ⁻¹)
<i>Patients with HeFH</i>					
1	Female	13.0	42.0	153.8	0.24
2	Female	7.9	29.4	125.9	0.34
3	Female	6.9	26.1	125.1	0.38
4	Female	9.7	28.4	139.7	0.35
5	Female	9.8	33.0	141.0	0.30
6	Female	13.4	45.3	160.5	0.22
7	Female	11.9	43.5	152.5	0.23
8	Female	6.0	19.2	114.1	0.52
9	Female	14.0	63.0	161.9	0.16
10	Female	15.6	57.8	168.4	0.17
11	Female	11.7	45.0	157.8	0.22
12	female	12.0	44.5	161.4	0.22
13	Female	12.0	50.3	152.7	0.20
14	Female	10.3	27.5	134.5	0.36
15	Male	6.3	20.5	119.2	0.49
16	Male	7.7	23.1	120.8	0.43
17	Male	10.3	48.8	151.8	0.20
18	Male	4.9	16.4	108.9	0.61
19	Male	10.7	56.6	145.2	0.18
20	Male	10.7	42.5	142.3	0.24
Mean ± SD		10.3 ± 2.9	38.1 ± 13.8	141.9 ± 17.7	0.30 ± 0.13
<i>Transplant recipients</i>					
21	Female	8.0	20.0	127.0	0.50
22	Female	18.7	46.3	160.0	0.22
23	Female	8.0	24.1	127.5	0.41
24	Male	11.6	42.5	146.0	0.24
25	Female	6.1	23.0	115.0	0.43
26	Male	13.8	40.3	143.0	0.25
27	Female	16.1	55.6	150.0	0.18
28	Female	4.4	15.5	103.0	0.65
29	Female	11.4	61.0	154.0	0.16
30	Female	16.9	71.0	163.0	0.14
31	Female	13.9	42.5	154.0	0.24
32	Fale	8.8	25.0	130.5	0.40
Mean ± SD		11.5 ± 4.5	38.9 ± 17.7	139.0 ± 18.8	0.32 ± 0.16

were healthy and none had received daily medication previously.

Of the 19 paediatric cardiac transplant recipients who had participated in our previous pravastatin study [10], 12 (nine girls and three boys) were studied again. Their mean age was 11.5 ± 4.5 years and their other characteristics are listed in Table 1. The immunosuppressive protocols included triple therapy with cyclosporin in microemulsion composition (12 patients), methylprednisolone (12 patients), and azathioprine (11 patients) or mycophenolate mofetil (one patient). Detailed information on the immunosuppressive regimen has been pub-

lished previously [10]. Additional medication on the day of the pravastatin pharmacokinetic study was the following: diltiazem ($n = 1$), felodipine ($n = 4$), nifedipine ($n = 1$), furosemide ($n = 4$), propranolol ($n = 2$), atenolol ($n = 1$), bisoprolol ($n = 1$), valganciclovir ($n = 2$), aciclovir ($n = 1$), and omeprazole ($n = 1$).

Study design

The protocol was approved by the ethics committee for paediatrics, adolescent medicine and psychiatry of the Helsinki and Uusimaa Hospital District. Written informed consent was obtained from the parents.

The patients in the two previous pharmacokinetic studies [2, 10] had ingested a single 10 mg dose of pravastatin (Pravachol, Bristol-Myers Squibb, Epernon, France) with 150 ml water after an overnight fast and did not eat earlier than 1.5 h after administration of pravastatin. The mean pravastatin doses kg^{-1} body-weight in the children with HeFH and in the transplant recipients were $0.30 \pm 0.13 \text{ mg kg}^{-1}$ and $0.32 \pm 0.16 \text{ mg kg}^{-1}$, respectively (Table 1). The transplant recipients ingested their morning medication with pravastatin. Plasma pravastatin concentrations had been determined by liquid chromatography-ionspray tandem mass spectrometry with use of the PE SCIEX API 3000 LC/MS/MS system (Sciex Division of MDS Inc, Toronto, Canada) [33] from samples taken before pravastatin and 0.5, 1, 1.5, 2, 3, 4, 8 and 10 h later. In the transplant recipients additional samples were taken at 12 and 24 h [2, 10]. The ion transition monitored was m/z 442 to m/z 269, and the limit of quantification was 0.25 ng ml^{-1} for pravastatin. The day-to-day coefficient of variation (CV) was 7.8% at 1 ng ml^{-1} ($n = 6$). After the pharmacokinetic study, all patients were treated with pravastatin 10 mg day^{-1} and blood lipids and safety parameters were monitored as described earlier [2, 10]. In the present genetic study, a 1 ml EDTA blood sample was drawn from each subject and stored at -20°C . DNA was extracted using standard methods (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany).

Pharmacokinetics and pharmacodynamics analysis

The pharmacokinetics of pravastatin were characterized by the peak concentration in plasma ($C_{\max}$), the time to peak concentration ($t_{\max}$), the elimination half-life ($t_{1/2}$), and the area under the plasma concentration-time curve from time 0 to infinity ($\text{AUC}(0, \infty)$) (in HeFH patients) [2] and from 0 to 24 h ($\text{AUC}(0, 24 \text{ h})$) (in cardiac transplant recipients) [10]. The pharmacokinetic software used was the MK Model (Biosoft, Cambridge, UK). The pharmacokinetics of pravastatin and the effect of 2 months daily pravastatin treatment on serum cholesterol and triglycerides in HeFH and transplant patients [2, 10] were evaluated in relation to the current genotyping results.

SLCO1B1 genotyping

All patients were genotyped for the $-11187\text{G} > \text{A}$ SNP in the promoter region and the $521\text{T} > \text{C}$ (Val174Ala) SNP in exon 5 of the *SLCO1B1* gene by allelic discrimination with TaqMan[®] 5' nuclease assays, using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Weiterstadt, Germany). The primers used in $521\text{T} > \text{C}$ genotyping were $5'$ -GAAACACTCTCTTA

TCTACATAGGTTGTTTA-3' (forward) and $5'$ -CCCC TATTCCACGAAGCAT-3' (reverse). The TaqMan[®] MGB probes were VIC-TACCCATGAACACATATA and FAM-TACCCATGAACGCATATA. The primers used for $-11187\text{G} > \text{A}$ were $5'$ -CATATATGCATCCTC ACATTACCACAT-3' (forward) and $5'$ -AATAAAGTAC AGACCCTTCTCTCACATAAA-3' (reverse), and the TaqMan[®] MGB probes were VIC-TGTATACAGGT AAAAGTG and FAM-TGTGTATACAAGTAAAG. The PCR conditions were one cycle at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles at 92°C for 15 s and at 60°C for 1 min, as recommended by the manufacturer. The accuracy of genotyping was confirmed by sequencing.

ABCB1 genotyping

All patients were genotyped for the $3435\text{C} > \text{T}$ SNP in exon 26 and the $2677\text{G} > \text{T/A}$ SNP in exon 21 of the *ABCB1* gene, by denaturing high-performance liquid chromatography [34]. *ABCB1* haplotype analysis was performed as described previously [35].

Statistical analysis

Results are expressed as mean values ($\pm$ SD) in the text and tables and, for clarity, as mean ($\pm$ SEM) in the figures. Statistical comparisons between two groups were made with Student's *t*-test for unpaired values. Differences in continuous variables between more than two groups were compared by one-way ANOVA with *a posteriori* testing using the Tukey test. $t_{\max}$ values were compared with the Mann-Whitney U-test or Kruskal-Wallis ANOVA with *a posteriori* testing using Dunn's test. StatsDirect (StatsDirect Ltd, Cheshire, UK) and SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA) were used for the statistical analyses. *P* values less than 0.05 were considered statistically significant.

Results

The *SLCO1B1* and *ABCB1* alleles and genotypes found in children with HeFH and in the cardiac transplant recipients are shown in Table 2. All observed genotype frequencies were consistent with the Hardy-Weinberg equilibrium.

Compared with 18 patients with the reference (wild type) genotype, two patients with the *SLCO1B1*-11187GA genotype (who also had the 521TC genotype) had an 81% lower mean $C_{\max}$ ($P < 0.001$) and a 74% lower $\text{AUC}(0, \infty)$ ($P < 0.0001$), and significantly greater increase in HDL-cholesterol after 2 months of pravastatin 10 mg day^{-1} ($26.7\% \pm 2.9\%$ vs. $3.2\% \pm 14.0\%$, $P = 0.0002$) (Table 3, Figure 1). Children with the *SLCO1B1* 521TC genotype ($n = 6$) had a 49% lower mean $C_{\max}$

Table 2

SLCO1B1 and *ABCB1* genotypes in 20 children with heterozygous familial hypercholesterolaemia (HeFH) and in 12 paediatric cardiac transplant recipients

Gene	Region	Position	Allele	Patients with HeFH <i>n</i> (%)	Transplant recipients <i>n</i> (%)	Genotype	Patients with HeFH <i>n</i> (%)	Transplant recipients <i>n</i> (%)
<i>SLCO1B1</i>	Promoter	-11187	G	38 (95%)	24 (100%)	GG	18 (90%)	12 (100%)
			A	2 (5%)	0 (0%)	GA	2 (10%)	0 (0%)
<i>SLCO1B1</i>	Exon 5	521	T	34 (85%)	21 (88%)	TT	14 (70%)	9 (75%)
			C	6 (15%)	3 (13%)	TC	6 (30%)	3 (25%)
<i>ABCB1</i>	Exon 21	2677	G	17 (43%)	15 (63%)	GG	4 (20%)	5 (42%)
			T	21 (53%)	8 (33%)	GT	8 (40%)	5 (42%)
			A	2 (5%)	1 (4%)	TT	6 (30%)	1 (8%)
						GA	1 (5%)	0 (0%)
<i>ABCB1</i>	Exon 26	3435	C	20 (50%)	13 (54%)	CC	5 (25%)	3 (25%)
			T	20 (50%)	11 (46%)	CT	10 (50%)	7 (58%)
						TT	5 (25%)	2 (17%)

Positions of SNPs correspond to their positions in the cDNA (exonic SNPs) or genomic sequence (promoter SNP) with the first base of the ATG first codon set to 1.

($P = 0.136$) and 26% lower AUC(0, ∞) ($P = 0.409$) of pravastatin than those with the reference genotype, but these differences were not statistically significant. Patient characteristics were similar in subjects with different *SLCO1B1* genotypes (Table 3). No significant differences existed in the lipid-lowering efficacy of pravastatin. Furthermore, there were no significant differences in the pharmacokinetics or effects of pravastatin between subjects with different *ABCB1* 3435C > T or 2677G > T/A genotypes or haplotypes (data not shown).

Cardiac transplant recipients with the *SLCO1B1* 521TC genotype ($n = 3$) had a 46% lower $C_{\max}$ ($P = 0.055$), a 62% smaller AUC(0,24 h) ($P = 0.016$) and a shorter $t_{1/2}$ (0.9 ± 0.1 vs. 1.3 ± 0.4 h, $P = 0.015$) of pravastatin than those with the reference genotype (Table 4, Figure 2). In transplant recipients with the 521TC genotype, the decreases in total ($-1.2\% \pm 5.8\%$ vs. $-18.9\% \pm 17.6\%$, $P = 0.031$) and LDL cholesterol ($-7.7\% \pm 7.7\%$ vs. $-33.5\% \pm 20.8\%$, $P = 0.011$) were smaller and the increase in HDL cholesterol was greater ($36.0\% \pm 15.6\%$ vs. $0.4\% \pm 23.0\%$, $P = 0.026$) than in those with the reference genotype (Table 4). No differences were apparent in patient characteristics between subjects with different *SLCO1B1* 521T > C genotypes (Table 4). There were no significant differences in the pharmacokinetics or effects of pravastatin between subjects with different *ABCB1* 3435C > T and 2677G > T/A genotypes or haplotypes (data not shown).

Discussion

To our knowledge, this is the first study to explore associations between the pharmacokinetics and efficacy of pravastatin (with and without immunosuppressive medication) and polymorphisms of the *SLCO1B1* and *ABCB1* genes in children. In patients with HeFH, the 521T > C SNP had no significant effect on the pharmacokinetics or efficacy of pravastatin, but two patients with the -11187GA genotype had significantly lower plasma concentrations of pravastatin than those with the reference genotype. Furthermore, the AUC(0,24 h) of pravastatin was markedly lower and the $t_{1/2}$ shorter in paediatric cardiac transplant recipients with the *SLCO1B1* 521TC genotype than in patients with the reference genotype. Decreases in total and LDL cholesterol by pravastatin were smaller, and increases in HDL cholesterol greater in association with the 521T > C SNP.

Recently, a number of SNPs in the *SLCO1B1* gene have been characterized. Increasing evidence suggests that some of these SNPs are functionally significant and decrease the activity of the encoded transporter OATP1B1 *in vitro* [36–39]. For example, Tirona et al. observed decreased uptake of oestrone sulphate, oestradiol 17 β -D-glucuronide and rifampicin by OATP1B1 in association with several *SLCO1B1* SNPs, including 521T > C [36, 37]. In addition, Kameyama et al. found reduced transport of pravastatin and atorvastatin acid in association with the 521T > C SNP [38]. Overall,

Table 3

Comparison of the baseline characteristics and the pravastatin pharmacokinetic and pharmacodynamic data in relation to the *SLCO1B1* 521T > C and -11187G > A single nucleotide polymorphisms in children with heterozygous familial hypercholesterolaemia

Variable	521TT (n = 14)	521TC (n = 6)	Difference in means (95% CI)	P	-11187GG (n = 18)	-11187GA (n = 2)	Difference in means (95% CI)	P
Age (years)	10.1 ± 3.2	10.6 ± 2.2	0.5 (-2.6, 3.5)		10.2 ± 2.8	10.1 ± 4.6	-0.1 (-4.7, 4.5)	
Weight (kg)	37.1 ± 15.0	40.6 ± 11.5	3.5 (-11.0, 17.9)		38.4 ± 14.2	35.7 ± 13.6	-0.7 (-24.9, 19.5)	
Height (cm)	140.2 ± 19.5	145.7 ± 13.7	5.5 (-13.0, 24.0)		141.8 ± 17.7	142.8 ± 25.0	1.0 (-27.5, 29.6)	
Body mass index (kg m ⁻²)	17.9 ± 3.1	19.0 ± 4.4	1.1 (-2.5, 4.6)		18.4 ± 3.6	17.2 ± 0.6	-1.2 (-6.7, 4.3)	
Pravastatin								
dose (mg kg ⁻¹)	0.32 ± 0.14	0.27 ± 0.08	-0.05 (-0.19, 0.08)	0.403	0.30 ± 0.13	0.30 ± 0.11	0.0 (-0.21, 0.20)	0.974
C _{max} (ng ml ⁻¹)	18.4 ± 15.6	9.4 ± 9.6	-9.0 (-21.0, 3.0)	0.136	17.1 ± 14.6	3.2 ± 0.1	-13.9 (-21.1, -6.7)	<0.001
AUC(0,∞) (ng ml ⁻¹ h)	34.5 ± 21.7	25.7 ± 20.0	-8.8 (-30.6, 13.0)	0.409	34.4 ± 20.7	9.1 ± 0.4	-25.3 (-35.6, -15.0)	<0.0001
t _{max} (h)	1.3 (0.5-4.0)	1.3 (1.0-1.5)		0.785	1.3 (0.5-4)	1.3 (1-1.5)		0.568
t _{1/2} (h)	1.6 ± 0.8	1.5 ± 0.2	-0.1 (-0.6, 0.4)	0.731	1.5 ± 0.8	1.7 ± 0.2	0.2 (-1.0, 1.3)	0.814
Change in cholesterol (mmol l ⁻¹)*	-1.3 ± 1.0	-1.4 ± 0.8	-0.1 (-1.1, 0.9)	0.869	-1.4 ± 1.0	-0.8 ± 0.8	0.6 (-0.9, 2.1)	0.401
Change in cholesterol (%)*	-15.3 ± 8.7	-18.6 ± 10.1	-3.3 (-12.5, 6.0)	0.466	-16.7 ± 8.7	-13.3 ± 14.8	3.4 (-11.0, 17.7)	0.631
Change in LDL-cholesterol (mmol l ⁻¹)*	-1.4 ± 1.0	-1.3 ± 0.6	0.1 (-0.9, 1.0)	0.908	-1.4 ± 1.0	-1.1 ± 0.7	0.3 (-1.2, 1.7)	0.702
Change in LDL-cholesterol (%)*	-20.1 ± 10.2	-23.2 ± 11.6	-3.1 (-14.0, 7.8)	0.554	-20.7 ± 9.8	-24.8 ± 20.4	-4.1 (-20.8, 12.5)	0.610
Change in HDL-cholesterol (mmol l ⁻¹)*	0.1 ± 0.1	0.0 ± 0.3	-0.1 (-0.3, 0.2)	0.548	0.0 ± 0.2	0.3 ± 0.0	0.3 (0.2, 0.4)	0.0001
Change in HDL-cholesterol (%)*	7.2 ± 12.5	1.7 ± 20.9	-5.5 (-24.7, 13.8)	0.572	3.1 ± 14.0	26.7 ± 3.0	23.6 (15.3, 31.7)	0.0002
Change in triglycerides (mmol l ⁻¹)*	-0.1 ± 0.6	-0.2 ± 0.4	-0.1 (-0.6, 0.5)	0.728	-0.2 ± 0.5	-0.1 ± 0.1	0.1 (-0.7, 0.9)	0.801
Change in triglycerides (%)*	-2.6 ± 35.7	-14.5 ± 24.4	-11.9 (-40.9, 17.0)	0.401	-6.1 ± 34.2	-6.2 ± 14.1	-0.1 (-52.4, 52.3)	0.999

Data are mean ± SD; t_{max} are median (range). *The change from baseline at 2 months with pravastatin 10 mg day⁻¹.

Figure 1

Plasma pravastatin concentrations (mean $\pm$ SEM) in relation to the *SLCO1B1* 521T > C (A) *SLCO1B1* 521TT subjects (n = 14) (○); *SLCO1B1* 521TC subjects (n = 6) (●) and -11187 G > A (B) *SLCO1B1* -11187GG subjects (n = 18) (○); *SLCO1B1* -11187GA subjects (n = 2) (●) single nucleotide polymorphisms in children with heterozygous familial hypercholesterolaemia

decreased transport activity of OATP1B1 is thought to lower the hepatic uptake of pravastatin from blood, and thus to impair its elimination and increase its plasma concentrations.

Recent *in vivo* studies in healthy adults have reported altered pharmacokinetics of pravastatin in association with polymorphisms in *SLCO1B1* [28–30]. One study observed decreased total and nonrenal clearance of pravastatin in healthy volunteers carrying the *SLCO1B1* haplotype *15 (388G and 521C) compared with those homozygous for *SLCO1B1**1B (388G and 521T) [28]. Another study reported significantly higher pravastatin AUC(0,12 h) in subjects with the -11187GA

Figure 2

Plasma pravastatin concentrations (mean $\pm$ SEM) in relation to the *SLCO1B1* 521T > C single nucleotide polymorphism in paediatric cardiac transplant recipients on a regimen of triple immunosuppression, *SLCO1B1* 521TT subjects (n = 9) (○); *SLCO1B1* 521TC subjects (n = 3) (●)

or 521TC genotypes or *15B or *17 (-11187 A, 388G and 521C) haplotypes [29], and a further study found increased pravastatin AUC(0,6 h) in heterozygous carriers of *SLCO1B1**5 (388 A and 521C), and decreased amounts of pravastatin excreted into urine in subjects heterozygous or homozygous for *SLCO1B1**1B, compared with subjects with the reference genotype [30]. As statins inhibit cholesterol synthesis in the liver, decreased uptake of pravastatin into hepatocytes due to defective transport might decrease its cholesterol-lowering efficacy. In support of this, a recent study showed that the inhibitory effect of pravastatin on cholesterol synthesis, evaluated by changes in lathosterol concentrations and lathosterol : cholesterol ratios, was smaller in patients with the *SLCO1B1* haplotype *17 [40]. Another investigation found significantly lower decreases in serum cholesterol during treatment with statins (pravastatin, atorvastatin or simvastatin) in 20 hypercholesterolaemic adults with the 521TC genotype, compared with 44 patients with the 521TT genotype (-16.5% vs. -22.3%) [41].

In contrast to a previous study in healthy adults [29], the mean pravastatin AUC(0, ∞) in two children with HeFH having the -11187GA genotype was approximately 74% lower than the corresponding value observed in patients with the reference genotype. Notably, no differences existed in the baseline characteristics between patients with different *SLCO1B1* genotypes. The mechanisms underlying these results are unclear and warrant further study. Very little is currently known

Table 4

Comparison of the baseline characteristics and the pravastatin pharmacokinetic and pharmacodynamic data in relation to the *SLCO1B1* 521T > C single nucleotide polymorphism in paediatric cardiac transplant recipients on a regimen of triple immunosuppression

Variable	<i>SLCO1B1</i> 521TT (<i>n</i> = 9)	<i>SLCO1B1</i> 521TC (<i>n</i> = 3)	Difference in means (95% CI)	P
Age (years)	11.6 ± 4.1	11.2 ± 6.6	−0.4 (−7.4, 6.7)	
Weight (kg)	41.4 ± 19.0	31.4 ± 12.9	−10.0 (−36.6, 16.7)	
Height (cm)	140.8 ± 18.6	135.2 ± 22.9	−5.6 (−34.6, 23.3)	
Body mass index (mg m ^{−2})	19.6 ± 5.2	16.7 ± 1.8	−2.9 (−9.9, 4.1)	
Cyclosporin dose (mg)	160 (135–300)	250 (150–300)		
Cyclosporin dose (mg kg ^{−1})	5.4 ± 2.0	7.6 ± 2.1	2.2 (−0.8, 5.2)	
Azathioprine dose (mg)	46.3 (18.8–75)	25 (25–75)		
Azathioprine dose (mg kg ^{−1})	1.2 ± 0.1	1.2 ± 0.3	0.0 (−0.4, 0.5)	
Azathioprine morning dose (mg)	21.88 (12.5–37.5)	12.5 (12.5–50)		
Pravastatin				
dose (mg kg ^{−1})	0.31 ± 0.17	0.35 ± 0.12	0.04 (−0.20, 0.29)	0.701
<i>C</i> _{max} (ng ml ^{−1})	145.7 ± 89.8	78.0 ± 10.3	−67.7 (−135.7, 0.3)	0.055
AUC(0,24 h) (ng ml ^{−1} h)	366.3 ± 223.5	137.8 ± 41.0	−228.5 (−402.7, −54.3)	0.016
<i>t</i> _{max} (h)	1.0 (0.5–2.0)	1.0 (1.0–1.5)		0.981
<i>t</i> _{1/2} (h)	1.3 ± 0.4	0.9 ± 0.1	−0.4 (−0.7, −0.1)	0.015
Change in cholesterol (mmol l ^{−1})*	−1.1 ± 1.2	−0.1 ± 0.3	1.0 (0.1, 2.0)	0.037
Change in cholesterol (%)*	−18.9 ± 17.6	−1.2 ± 5.8	17.7 (1.9, 32.1)	0.031
Change in LDL-cholesterol (mmol l ^{−1})*	−1.0 ± 0.8	−0.2 ± 0.3	0.8 (0.1, 1.5)	0.022
Change in LDL-cholesterol (%)*	−33.5 ± 20.8	−7.7 ± 7.7	25.8 (7.4, 44.2)	0.011
Change in HDL-cholesterol (mmol l ^{−1})*	0.0 ± 0.4	0.4 ± 0.2	0.4 (0.1, 0.7)	0.032
Change in HDL-cholesterol (%)*	0.4 ± 23.0	36.0 ± 15.6	35.6 (10.0, 62.7)	0.026
Change in triglycerides (mmol l ^{−1})*	−0.1 ± 0.8	−0.6 ± 0.6	−0.5 (−1.6, 0.7)	0.358
Change in triglycerides (%)*	−2.5 ± 39.0	−27.1 ± 15.1	−24.6 (−59.5, 10.2)	0.149

Data are mean ± SD; cyclosporin dose (mg), azathioprine doses (mg) and *t*_{max} are median (range). *The change from baseline at 2 months with pravastatin 10 mg day^{−1}.

about the potential role of developmental characteristics affecting the pharmacokinetics of statins in childhood or about age-associated differences in the expression and function of drug transporters [42]. The present results suggest that the effects of *SLCO1B1* polymorphism on OATP1B1 phenotype may be modulated by age.

In paediatric cardiac transplant recipients, the 521T > C SNP was associated with significantly decreased plasma concentrations of pravastatin and with a shorter half-life, despite similar baseline characteristics among the patients. It is unclear why the plasma concentrations of pravastatin were decreased. Along with the low plasma concentrations of pravastatin in our cardiac transplant recipients with the 521TC genotype, the total and LDL cholesterol-lowering effects in these patients were less, suggesting that hepatocellular pravastatin concentrations were also decreased.

Cyclosporin is known to cause substantial increases

in the plasma concentrations of pravastatin [10, 18, 19], through mechanisms that are not completely understood. As cyclosporin inhibits drug transporters such as P-glycoprotein [43], MRP2 [17] and OATP1B1 [16], the interaction could be due to inhibition of pravastatin transport. In our previous pharmacokinetic study in paediatric cardiac transplant patients, immunosuppressive medication increased pravastatin *C*_{max} 8-fold and AUC 10-fold, but left the *t*_{1/2} unaffected [10].

The allelic frequencies of the *SLCO1B1* 521C variant (15% and 13%) in children with HeFH and paediatric cardiac transplant recipients were similar to those found in Caucasian and Japanese adult populations (11–18%) [28,29]. The respective frequencies of the promoter variant (−11187 A) were also similar, though slightly smaller than those previously reported in Caucasians (5% and 0% vs. 7.5%) [29]. The allelic frequencies of the ABCB1 3435T (50% and 46%), 2677T (53% and

33%) and 2677 A (5% and 4%) variants were also comparable with those previously reported in adult Finnish Caucasians (55%, 48% and 1%, respectively) [29].

The present results suggest that differences may exist in the pharmacogenetics of pravastatin between children and adults. Larger trials are required to assess the underlying mechanisms and clinical significance of these findings. Because undertreated hypercholesterolaemic patients are likely to remain at an increased risk of cardiovascular morbidity and mortality, further characterization of the factors affecting the pharmacokinetics of statins is important in both adults and children.

In conclusion, in children with HeFH, the *SLCO1B1*-11187GA genotype was unexpectedly associated with markedly decreased plasma concentrations of pravastatin. Similarly, cardiac transplant recipients undergoing triple immunosuppressive therapy had lower plasma concentrations and a shorter half-life of pravastatin in association with the *SLCO1B1* 521T > C SNP. Owing to the small number of subjects included, these results require substantiation in larger trials.

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