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ORIGINAL ARTICLE

Cytochrome P450 2D6 polymorphism and drug utilization in patients with oral lichen planus

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Abstract

Objective. Oral lichen planus (OLP) is one of the commonest diseases of the oral mucosa. The etiology of the disease is unknown. Our goal was to determine frequencies of functionally important alleles which determine the metabolic rate (phenotype) of individuals with OLP and to compare drug utilization, with focus on CYP2D6, with that of a control group. ***Material and methods.*** The study population consisted of 46 patients with OLP, 60 sex- and age-matched control subjects for drug utilization evaluation and 223 healthy non-medicated controls for genotype comparison. DNA analysis was done using polymerase chain reaction and restriction fragment length polymorphism. The gene CYP2D6 was analyzed for the alleles CYP2D6*3,*4,*5,*6 and gene duplication. Drug utilization was evaluated according to Anatomical Therapeutic Chemical code, liver drug metabolism pathway and mono- or polytherapy. ***Results.*** Intake of drugs was significantly higher in the group of OLP patients in comparison with control subjects. The use of CYP2D6 substrates, inhibitors or inducers did not differ between OLP patients and controls. Predicted phenotype frequencies in OLP patients and healthy controls, respectively were as follows: ultrarapid metabolizers 2% and 5.8%, extensive metabolizers 52% and 49.8%, intermediate metabolizers 39% and 37.7% and poor metabolizers 7% and 6.7%. ***Conclusions.*** We did not find a statistically significant difference in the frequency of CYP2D6 alleles between OLP patients and healthy controls. OLP patients used more medication than age- and sex-matched controls.

Key Words: *CYP2D6, cytochrome P450, genetic polymorphism, oral lichen planus, pharmacotherapy*

Introduction

Lichen planus is a chronic inflammatory disorder of the skin and mucosal surfaces. Oral lesions known as oral lichen planus (OLP) very often develop during the course of the disease. The prevalence of OLP in the general population is considered to be 1%–2% [1–4]. The disease usually manifests at the age of 50–70 years, and is very rare in children [5]. Women are more often affected than men (2:1.5) [5]. White lesions of various appearances can commonly be found upon examination.

Lesions are most often found on the buccal mucosa (≈90%), tongue (30%) and gingiva (13%). Occasionally they can also be found on the lips and palate [2,6]. Typically, the appearance of lesions may change during the course of the disease [2].

Although the etiology and pathogenesis of OLP have been extensively studied, they still remain unknown [7]. An etiopathogenetic connection to systemic diseases is a matter of discussion. Sometimes the etiology is believed to be known and some theories describe an association with hepatitis C [8–11]. Contact reactions to dental amalgam [12,13], stress [14,15] or drug exposure have been reported to initiate OLP. Oral lichenoid drug reactions may be triggered by the use of almost any drug, such as non-steroidal anti-inflammatory drugs (NSAIDs), beta-blockers, sulfonylurea derivatives, angiotensin-converting enzyme (ACE) inhibitors or antimalarics [16–18]. Almost all

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observations are however based on case reports. Many drugs which are believed to be pathogenetically associated with OLP are metabolized by cytochrome P450 (CYP450). This enzyme system catalyzes oxidative biotransformation reactions that lead to excretion of xenobiotics from the organism and prevent their accumulation.

CYP2D6 shows significant polymorphism, leading to predicted poor, extensive and ultrarapid metabolism of substrates for this isoenzyme [19]. Therefore the drug substrates of CYP2D6 may have different safety and efficacy profiles in patients belonging to different CYP2D6 phenotype groups [19]. Therefore we performed this study in order to clarify whether CYP2D6 polymorphism represents an independent risk factor for developing OLP. In parallel we wanted to evaluate the drug utilization habits of OLP patients in order to address possible exogenic factors associated with CYP2D6 activity.

Material and methods

Three subsets of patients were created: one OLP patient group and two control groups, one of which was used for comparison of utilization of medicines and the other served for genotype comparison. The experimental group consisted of 46 patients diagnosed with OLP (34 females, 12 males; mean age 65 years). These were consecutive patients visiting to our dentistry department, and no other selection procedure was performed. All patients were Caucasian and of Czech nationality. The study was approved by the local ethical committee. All participants signed an informed consent form before entering the study. The patients were examined and followed up at the Department of Dentistry of the Faculty of Medicine and University Hospital in Hradec Králové.

The diagnosis of OLP was determined by the typical clinical signs of the disease. Three different types of lesion were recognized, in line with previously published data [20]:

(1) Reticular form (characterized by mucosal keratosis).
(2) Atrophic form (where keratosis is combined with epithelial atrophy).
(3) Ulcerative form (where keratosis is combined with shallow ulcerations caused by epithelial necrosis and red and white mucosal changes found in the previous forms).

Histopathologic examination was done for all lesions to confirm the diagnosis. Findings had to be consistent with OLP: hyperparakeratosis of the epithelial layer and acanthosis; a dense lymphocytic infiltrate in the basal membrane zone; and degenerated or apoptotic keratinocytes (so-called 'Civatte bodies') [21–23].

Patients with suspected contact lesions were excluded from the study. A pharmacological history was taken from all the OLP patients to determine all drugs administered, and a sample of venous blood for DNA analysis was taken. The control group for drug utilization consisted of 60 subjects (43 females; 17 males) who consecutively visited the dental clinic within the study period without any records or current signs of mucosal disorders. Control subjects were matched by age and sex. Reviewed medical records included information on personal history, abuse habits and medication, which was evaluated by a standard questionnaire and an interview with the dentist. Drug utilization was evaluated according to the Anatomical Therapeutic Chemical (ATC) code, liver drug metabolism pathway and mono- or polytherapy. Identical procedures for drug utilization were performed for the OLP patient group and the drug utilization control group. The control group for genotype comparison consisted of 223 healthy volunteers who had been examined for the CYP2D6 polymorphism in our previous study conducted in 2006 [24] (89 females, 134 males; mean age 24 years). All control subjects for genotyping were healthy, with no concomitant disease and took no medication. The technique of sample processing was the same in both the OLP patient group and genotype control group, while the analysis was conducted by the same laboratory. DNA was isolated by a standard phenol–chloroform method and analyzed by polymerase chain reaction (PCR) and PCR–restriction fragment length polymorphism according to the procedure published by Buzková et al. [24]. Alleles of CYP2D6*3, *4, *5 and *6 and gene duplication were detected. When none of these alleles were detected, the presence of CYP2D6*1 was assumed. The phenotypes of ultrarapid, rapid, intermediate and poor metabolizers were then predicted for each genotype, as follows: ultrarapid metabolizers for carriers of CYP2D6*1 duplication; extensive metabolizers for carriers of CYP2D6*1/CYP2D6*1; intermediate metabolizers carrying one of the variant alleles CYP2D6*3/*4/*5/*6; and poor metabolizers (PM) being homozygous for the variant alleles CYP2D6*3/*4/*5/*6.

Statistical analysis was done using Microsoft Excel 8.0 (Microsoft, Seattle, WA) and Statgraphics Plus 3.1 (StatPoint Inc., Warrenton, VA) software. The occurrence of allelic frequencies and phenotypes was compared using the χ^2 test. $p < 0.05$ was considered statistically significant.

Results

Four patients were diagnosed with the ulcerative form of OLP and 42 had the reticular form. Drug intake was significantly higher in the group of OLP patients in comparison with the control subjects. Mean drug

Table I. Distribution of subjects according to sex and number of drugs used daily.

	Number of medications used daily			
Subjects	0	1	2–4	>4
OLP				
Men (n = 12)	1	1	5	5
Women (n = 34)	2*	3	11	18
Total (n = 46)	3**	4	16	23
Control group				
Men (n = 17)	5	1	5	6
Women (n = 43)	14	5	13	11
Total (n = 60)	19	6	18	17

*$p < 0.05$; **$p < 0.01$ vs control group.

consumptions were 4.5 and 3.1 medicaments per day per person in the patients and controls, respectively. Approximately 93% of the patients (43/46) reported daily intake of one or more drugs, while only 68% of control subjects (41/60) were regularly treated. The distribution of drug users with respect to the number of different medications is shown in Table I. There was a tendency towards more frequent polymedication in the group of patients in comparison with control subjects, but the difference was not significant. We further noted no substantial difference in drug utilization patterns between men and women within either the patient or control groups.

We identified 147 individual active compounds regularly used by at least one subject in either group. The ATC classification of the most frequently used compounds is summarized in Table II. Medications for cardiovascular diseases were used in approximately two-thirds of the patients. Patients of either sex frequently used hypolipidemics, antidiabetics, diuretics, beta-blockers, ACE inhibitors and antithrombotics. NSAIDs and anxiolytics were disproportionately more frequently used by female patients in comparison with males. On the other hand, venoprotective agents were more characteristic for male patients with OLP than for females. Only 12 of the total of 147 active compounds were found to be substrates for the liver CYP2D6 metabolic pathway (Table III). The use of these compounds was considerably low and the only drug that was more frequently used by the patients with OLP in comparison with control subjects was the analgesic compound tramadol. Table IV shows the numbers of subjects in each group receiving CYP2D6 and non-CYP2D6 substrates. Consistent with the CYP2D6 substrate consumption in the study population, there was no significant difference between the numbers of CYP2D6 substrate-using subjects. The odds ratio for OLP in patients consuming CYP2D6 substrates was 0.68 (95% confidence interval 0.31–1.50) in relation to the control subjects.

Frequencies of the variant alleles in the OLP patients were 0% for CYP2D6*6, 4% for CYP2D6*5, 22% for CYP2D6*4, 2% for CYP2D6*3 and 2% for gene duplication. The corresponding frequencies in the control group were 0.2%, 3.1%, 22.9%, 1.1% and 3.1%, respectively. Genotype distribution and predicted phenotypes are shown in Table V. The distribution of the alleles was in Hardy–Weinberg equilibrium in both groups. None of the genotype differences between the OLP patient group and the healthy control group were statistically significant (Table III).

Discussion

The design of our study comprised two control groups to allow testing of our hypothesis with two

Table II. Number of OLP and control subjects taking medications classified into the seven ATC groups most frequently used by men or women with OLP.

	OLP patients			Control group		
ATC group	Men	Women	Total	Men	Women	Total
C10 hypolipidemics	6	12*	18**	3	6	9
A10 antidiabetics	4*	12	16**	0	8	8
C03 diuretics	4	12	16	3	19	22
C07 beta-blockers	6	9	15	10	12	22
C09 ACE inhibitors	5	9	14	8	14	22
M01 NSAIDs[a]	1	12**	13**	3	0	3
B01 antithrombotics	5	7	12	5	11	16
N05 anxiolytics[a]	1	9*	10*	0	5	5
C02 venoprotectives[b]	4*	2	6	0	2	2

*$p < 0.05$; **$p < 0.01$ vs control group.
[a]ATC group frequently used by women with OLP only.
[b]ATC group frequently used by men with OLP only.

Table III. Numbers of patients in the OLP and control groups using CYP2D6 substrate drugs.

	OLP patients			Control subjects		
Drug (ATC code)	Men	Women	Total	Men	Women	Total
Betaxolol (S01)	2	5	7	3	4	7
Carvedilol (C07)	0	1	1	2	0	2
Citalopram (N06)	0	1	1	0	2	2
Flupentixol (N05)	0	1	1	0	0	0
Formoterol (R03)	0	0	0	0	1	1
Loratadine (R06)	0	0	0	0	1	1
Metoclopramid (A03)	1	1	2	0	0	0
Metoprolol (C07)	3	3	6	5	3	8
Paroxetine (N06)	0	1	1	0	0	0
Tamoxifen (L02)	0	0	0	0	1	1
Tolterodine (G04)	0	1	1	0	0	0
Tramadol (N02)	1	2	3*	0	0	0
Total	7	16	23	10	12	22

*$p < 0.05$ vs control group.

independent susceptibility factors, drug utilization and CY2D6 genotype, that possibly trigger OLP. The drug utilization of OLP patients was compared with that of age- and sex-matched control subjects visiting dental clinics who had no signs of mucosal damage. Since other frequent diseases, such as hypertension or other systemic diseases, did not represent exclusion criteria for the drug utilization control subjects, we needed to compare the allelic and genotype frequencies with those of healthy subjects to avoid bias that may result from a possible unknown deviation of the distribution of variant alleles in the control group. Therefore data from healthy, unrelated Czech volunteers were used for genotype comparison.

Although the etiopathogenesis of OLP is complex and poorly understood, interactions with drug usage and environmental and lifestyle factors have been reported. The OLP patients in this study consumed a significantly higher number of daily medications, especially from the ATC groups of hypolipidemics, antidiabetics, NSAIDs and anxiolytics, compared to control subjects. The mean age of the OLP patients was 61 years and mean daily drug consumption was 4.5 individual medicaments. Such a level of prescribed drug utilization corresponds to that of considerably older subjects in the general population of the Czech Republic, where an average intake of four to five medications is observed in subjects aged >75 years. From this perspective our results may support some involvement of polymedication in the development of OLP, although the association is weak. This high utilization of drugs may also raise the potential for drug–drug interactions, although we have not observed substantially different usage of CYP2D6 inducers of inhibitors between the study populations. Interestingly, substantially more OLP patients (93%) in the present study consumed drugs than in some previously published studies [18,25,26], in which regular drug intake was reported by up to 50% of OLP patients. This may, however, reflect the generally high level of underreporting of drug use in dental clinics or may relate to the different socioeconomic status of the subjects in different studies because ≈68% of the control subjects in our study reported regular drug use.

Table IV. Numbers of subjects receiving treatment metabolized via CY2D6 and other drug-metabolizing enzymes.

Group	Non-CYP2D6 substrate	CYP2D6 substrate medicated subjects	Total no. of
OLP			
Men	6	5	11
Women	19	13	32
Total	25	18	43
Controls			
Men	2	10	12
Women	18	11	29
Total	20	21	41

It has previously been suggested that the use of medications metabolized by polymorphic cytochrome P450 enzymes may be implicated in the development of OLP [17,18], although no individual hemoproprotein has been identified. Our study focused on CYP2D6 substrates, since polymorphism of this hemoprotein is considered of high importance among liver drug-metabolizing P450 cytochromes. The P450 cytochromes are predominantly expressed in the liver, but their extra-hepatic localization in oral mucous membrane has also been observed. CYP2D6 expression in buccal tissue has been shown

Table V. Frequencies of CYP2D6 genotypes and predicted phenotypes in the OLP and control groups.

	OLP group (%)	Control group (%)
Extensive metabolizers CYP2D6*1/CYP2D6*1	52	52
Intermediate metabolizers	39	37
CYP2D6*1/CYP2D6*4	30	31.4
CYP2D6*1/CYP2D6*5	7	4.5
CYP2D6*1/CYP2D6*3	2	1.8
Poor metabolizers	7	7
CYP2D6*4/CYP2D6*4	5	5.3
CYP2D6*4/CYP2D6*5	2	0.4
CYP2D6*4/CYP2D6*3	0	0.4
CYP2D6*4/CYP2D6*6	0	0.4
Ultrarapid metabolizers CYP2D6*1x2/CYP2D6*1	2	4

in 6/13 patients [27], suggesting high inter-individual variability of the local metabolic fate of CYP2D6 substrates, which could be a reflection of the inherent polymorphism of this enzyme. We have not observed markedly different patterns of CYP2D6 substrate/inducers/inhibitors utilization between OLP and control subjects, although there was a tendency towards higher CYP2D6 substrate use. However, this observation is not necessarily in conflict with the results of higher consumption of drugs with PM risk (e.g. substrates of CYP2C9, CYP2C19 and CYP2D6) published by Kragelund et al. [18], because many compounds from the ATC groups that were more frequently used by OLP patients in our study belong among the polymorphic CYP2C9 substrates (mainly antidiabetics and NSAIDs).

The distributions of CYP2D6 genotypes and allelic frequencies in patients with OLP were similar to those of the healthy control group and the data are consistent with the known genotype distribution in other healthy European populations [24]. We screened our subjects only for the most frequent variant alleles within the CYP2D6 gene. The other known functionally deficient alleles CYP2D6*8, *9 and *10 occur in our population with an allelic frequency of ≈1% (own unpublished data). This implies that the frequency of homozygous PM for each of these alleles would be ≈0.01%, which is too small a figure given the size of our study group. There is no difference in the CYP2D6 variant allele distribution between men and women, but the incidence of OLP is generally higher in women. This aspect is of note in connection with our observation of significantly fewer female OLP patients without any regular medication intake. This could suggest that different drug utilizations between males and females may be a risk factor for OLP. However, there may be other unknown factors that determine the sex-dependent incidence of OLP.

Our study did not reveal any statistically significant difference in CYP2D6 polymorphism between OLP patients and controls. From this point of view, we failed to show a potential connection between OLP and CYP2D6.

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