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Impact of switching to a heat-not-burn tobacco product on CYP1A2 activity

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ABSTRACT

Background: Cigarette smoking induces cytochrome P450 1A2 (CYP1A2) expression and activity, while smoking cessation normalizes the levels of this enzyme. The aim of this publication is to summarize the data on CYP1A2 gene expression and activity in preclinical and clinical studies on the Tobacco Heating System (THS), currently marketed as *IQOS*® with *HEETs*®, and to summarize the potential effects on CYP1A2 to be expected upon switching to reduced-risk products (RRPs).

Methods: We summarized PMI's preclinical and clinical data on the effects of switching from cigarette smoking to THS.

Results: Data from four preclinical mouse and rat studies showed that, upon either cessation of cigarette smoke exposure or switching to THS exposure, the upregulation of CYP1A2 observed with exposure to cigarette smoke reverted close to fresh-air levels. Data from four clinical studies yielded similar results on CYP1A2 activity within a time frame of five days. Furthermore, the effects of switching to THS were similar to those seen after smoking cessation.

Conclusions: Because smoking cessation and switching to either electronic cigarettes or THS seem to have similar effects on CYP1A2 activity, the same measures taken for patients treated with narrow therapeutic index drugs that are metabolized by CYP1A2 and who quit smoking should be recommended for those switching to RRPs.

1. Introduction

The cytochrome P450 (CYP) family of mono-oxygenases are important enzymes involved in the metabolism of drugs, pesticides, and endogenous metabolites [1]. Of these, human CYP1A enzymes should be considered not only in pharmacology but also in physiopathology, as they are involved in the detoxification of drugs as well as metabolic activators of harmful xenobiotics, such as aromatic amines and heterocyclic aromatic amines, that have carcinogenic potential [2,3]. There are two CYP1A isoforms expressed in humans out of which CYP1A2 is preferentially expressed and accounts for 13–15 % of the total hepatic CYP content, while CYP1A1 is considered an extrahepatic enzyme in humans [2–4]. Furthermore, there is no evidence of expression of CYP1A2 in extrahepatic tissues [5].

CYP1A2 is involved in the metabolism of about 9% of marketed drugs [3], such as theophylline [6], propranolol [7], verapamil [8], and clozapine [9], among others. Plasma concentrations of such drugs, therefore, depend on the activity of this enzyme [10]. Because some of the medications metabolized by CYP1A2 have a narrow therapeutic index (e.g., theophylline and clozapine), actual individual enzyme activity may have an important effect on their efficacy and tolerability, and a correspondingly individualized dose is often required for such drugs.

Large inter- and intra-individual variability of CYP1A2 activity has been observed in humans [11]. This variability is partly because CYP1A2 is induced or inhibited by other drugs, environmental compounds, and dietary or lifestyle-related factors. Cigarette smoking, for instance, has been shown to induce CYP1A2 activity, which has been quantified to be 1.72-fold higher in heavy smokers (≥20 cigarettes per day) compared to nonsmokers [12]. Additionally, sudden smoking cessation and the subsequent abolishment of CYP1A2 induction could lead to adverse drug reactions [10]. Case reports describing adverse reactions caused by elevated concentrations of different drugs after smoking cessation suggest that dose adjustment should be conducted whenever patients cease smoking while under treatment with CYP1A2-metabolized drugs, particularly those with narrow therapeutic

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indices [13–17]. Specifically, it is the exposure to polycyclic amino hydrocarbons (PAHs) which are products of incomplete combustion of organic matter through tobacco smoking that results in the induction of drug-metabolizing enzymes [2]. Because the effect on hepatic microsomal enzymes is not related to the nicotine component of tobacco, nicotine replacement will not alter this effect [18].

Currently, Philip Morris International (PMI) has a range of reduced-risk products (RRPs) at various stages of development, scientific assessment, and commercialization. RRPs are defined as products that present, are likely to present, or have the potential to present less risk of harm to adult smokers who switch to these products versus continuing smoking. One such product is the Tobacco Heating System (THS), currently marketed as *IQOS*® with *HEETS*®, which was launched in November 2014 in Japan and is currently marketed in over 52 countries. In order to substantiate the risk-reduction potential of RRPs, including the THS, PMI has implemented an assessment program which follows the United States Food and Drug Administration's (FDA) Modified Risk Tobacco Product Application (MRTPA) draft guidance [19].

PMI's assessment studies have evaluated both CYP1A2 gene expression and activity because switching to the THS has been shown to reduce exposure to harmful and potentially harmful constituents (HPHCs) found in cigarette smoke (CS)—including PAHs such as benzo[*a*]pyrene (B[*a*]P)—by an average of 90 % [20,21]. B[*a*]P has been shown by Vu et al. [22] to be a suitable marker for estimating the overall emission of PAHs generated during tobacco combustion, as correlation analyses show a strong positive and linear associations between B[*a*]P and most other PAHs as well as the overall PAH content in any cigarette brand [22,23]. It has also been shown that PMI's methods to measure B[*a*]P [22] provide the same data as those published by Vu et al. [25]. Therefore, B[*a*]P is a suitable surrogate marker for all PAHs found in CS and THS aerosol. Emission of B[*a*]P has been shown to be considerably lower in THS aerosol than in smoke from the 3R4F standard reference cigarette (<1.00 to 1.29 ± 0.10 ng/stick vs. 14.2 ± 0.3 ng/cigarette) [21].

The present article summarizes the effects of switching to the THS on exposure to PAHs (3-hydroxybenzo[*a*]pyrene or 3-OH-B[*a*]P) as a measurement of B[*a*]P) as well as CYP1A2 gene expression and activity in preclinical and clinical studies conducted by PMI.

2. Methods

2.1. Preclinical studies

Within the systems toxicology assessment framework for RRPs, PMI conducts comprehensive molecular profiling experiments, using microarray-based transcriptomics and mass-spectrometry based proteomics for mouse and rat exposure studies. In this context, PMI has evaluated the changes in CYP1A2 protein and mRNA expression in the liver in two ApoE$^{-/-}$ mouse studies [24,25] and two Sprague Dawley rat exposure studies [26–28].

In the mouse studies, female ApoE$^{-/-}$ mice were exposed for up to six or eight months to 3R4F CS or THS aerosol at matched nicotine concentrations [24,25]. The rat studies represent 90-day inhalation toxicology studies conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline 413 and complemented with systems toxicology endpoints. The first rat study (OECD 90-day rat THS) assessed the effects of THS aerosol [28], and the second study (OECD 90-day rat THS mentholated) assessed the effects of THS mentholated aerosol [26,27]. In addition to 3R4F CS, the second study also included CS from mentholated reference cigarettes with two different menthol concentrations (1XMIS and 2XMIS). In addition, the ApoE$^{-/-}$ mouse studies included cessation (CESS) groups, exposing the mice for two or three months to 3R4F CS before changing the exposure to fresh air. The first ApoE$^{-/-}$ mouse study also included a switching arm from 3R4F CS to THS aerosol group (SWITCH).

2.2. Clinical studies

PMI evaluated CYP1A2 activity in four randomized, controlled, open-label three-arm studies. Two studies evaluated smokers switching from cigarette smoking (CC) to THS regular or smoking abstinence (SA) compared to smokers continuing to smoke for five days in confinement (Poland and Japan) [29,30]. The other two studies evaluated smokers switching from smoking mentholated cigarettes (mCC) to THS menthol or SA compared to smokers continuing to smoke for five days in confinement, followed by up to 86 days in an ambulatory setting (Japan, US) [31,32].

The five-day studies conducted in Poland (NCT01959932) [30] and Japan (NCT01970982) [29] evaluated the changes in CYP1A2 activity on day five in smokers switching from combustible cigarettes (CC) to THS regular or SA and continuing to smoke regular cigarettes.

The 90-day studies conducted in Japan (NCT01970995) [31] and the U.S. (NCT01989156) [32] evaluated the changes in CYP1A2 activity in smokers switching from mentholated cigarettes to THS menthol as compared to smokers continuing mCC and SA.

For all studies, all CYP1A2 summaries and analyses were performed in subjects compliant with the protocol requirements in terms of adherence to allocated arm and absence of major deviations that would influence the evaluability of the primary objectives of the study. Summary descriptive statistics, including arithmetic means and percentage of change from baseline, were estimated. In the two five-day studies, arithmetic means in the THS, CC, and SA arms and LS Mean Difference between the THS and CC arms and between the THS and SA arms were computed. In the two 90-day studies, an assumption of log-normal distribution of CYP1A2 enzymatic activity was made, and geometric means and LS Mean ratios (THS menthol:mentholated cigarettes) and (THS menthol:SA) were estimated. However, to ensure comparability of results across studies, arithmetic means are presented in this article.

2.3. CYP1A2 activity test

The measurement of enzyme activity was assessed through paraxanthine (PX) and caffeine (CAF) plasma molar concentration ratios approximately six hours (±15 min) after intake of either a cup of coffee made from 4.2 g (±10 %) of regular instant coffee (Nescafé Gold Instant, Nestlé, Germany; CAF content: 72 mg/2 g) with 150 ± 10 mL water (five-day study in Poland) or after intake of one Tomerumin® CAF tablet with 150 ± 10 mL of water (five-day and 90-day studies in Japan). The tablet contained around 170 mg CAF [33], and the cup of coffee had a CAF content of approximately 150 mg [10]. In the 90-day study in the U.S., Vivarin® CAF tablets (Meda Consumer Healthcare Inc., USA; CAF content: ~200 mg) were taken with 240 ± 10 mL water. The exact time of coffee or CAF intake in the morning and blood sampling (six hours [±15 min] after intake of coffee) were recorded. Cytochrome P450 1A2 activity was assessed by measuring the molar PX/CAF metabolic ratio [10].

2.4. Measurement of exposure to B[a]P

Total 3-OH-B[*a*]P (free or sulfate and glucuronide conjugates) represents about 40 % of the metabolite fraction of inhaled B[*a*]P, one of the PAHs present in tobacco smoke [34]. Total 3-OH-B[*a*]P concentration was measured in the 24 -h urine samples collected from the study participants. The analysis was performed by liquid chromatography–mass spectrometry (LC–MS/MS) after enzymatic hydrolysis. The levels were normalized over urinary creatinine levels, which were measured spectrophotometrically.

3. Results

3.1. Preclinical studies

The studies assessing protein and mRNA expression changes for CYP1A2 in liver across two ApoE$^{-/-}$ mouse and two Sprague Dawley rat exposure studies are described in Table 1. The ApoE$^{-/-}$ mouse model was chosen for the preclinical assessment studies because it allows for the concomitant assessment of respiratory and cardiovascular endpoints [35], while – to our knowledge – no effect of the ApoE$^{-/-}$ genotype on CYP1A2 responses has been reported. Female mice were selected to reduce the number of animals needed for testing, in the context of the "3Rs" (Reduce, Refine, Replace), while focusing on the more susceptible sex for respiratory endpoints [36–38].

In three of these four *in vivo* studies, CS exposure resulted in significant upregulation of CYP1A2 levels. The fold changes in CS-exposed animals compared to fresh air-exposed animals remained relatively low (maximum of 1.76 for protein and 1.45 for mRNA expression changes). In the context of these low fold changes, it is important to note that the lack of significance for some CS-exposed groups with similar effect sizes can be due to the analytical variability and multivariate nature of these assays. In particular, the detection of the CYP1A2 response appeared more robust in the mouse than in the rat studies – with only three of twelve CS to Sham comparisons found significant in only one of the evaluated rat studies (OECD 90-day rat THS2.2 M). In contrast to CS, THS (M) (THS menthol) aerosol exposure did not result in differential expression of CYP1A2 in any of the four studies. The effects of cessation and switching to the THS after two months (ApoE$^{-/-}$ mouse study #1) or three months (ApoE$^{-/-}$ mouse study #2) of CS exposure were also evaluated in these studies, with the selection of the cessation/switching time point guided by the observed rapid onset of inflammation and emphysematous alterations upon CS exposure in mice [39–41]. Upon both cessation and switching to THS, the upregulation of CYP1A2 observed upon CS exposure reverted close to fresh air levels.

3.2. Clinical studies

The descriptive characteristics of the study participants are presented in Table 2, and a summary of the descriptive statistics for each arm for CYP1A2 activity is presented in Table 3. The percent changes from baseline CYP1A2 levels are presented in Table 4, and those of the urinary levels of total 3−OH-B[*a*]P are presented in Table 5.

Table 2
Demographic characteristics of the clinical study populations (per protocol populations).

	5-day study in EU	5-day study in JP	3-month study JP	3-month study U.S.*
Number of participants (N)				
THS arm	80			
CC arm	41	40	42	35
SA arm	39	40	39	24
Sex (%)				
THS arm (men/women)	48.8/51.3	50/50	56.6/43.4	61.3/38.7
CC arm (men/women)	51.2/48.8	50/50	59.5/40.5	57.1/42.9
SA arm (men/women)	51.3/48.7	50/50	56.4/43.6	62.5/37.5
Age [mean (SD)], years				
THS arm	35.4 (9.40)	37.6 (11.7)	37.2 (10.7)	39.0 (11.8)
CC arm	32.6 (10.06)	37.2 (11.7)	37.4 (11.2)	34.1 (10.5)
SA arm	33.6 (11.51)	35.9 (10.6)	37.4 (11.2)	40.5 (10.8)
BMI [mean (SD)], kg/m^2				
THS arm	24.46 (3.034)	22.75 (2.670)	22.80 (2.905)	27.19 (4.136)
CC arm	25.80 (3.228)	22.88 (2.667)	22.44 (2.876)	26.06 (3.805)
SA arm	24.81 (2.505)	22.88 (2.667)	22.53 (3.411)	25.79 (3.240)

Table 1
CYP1A2 expression in mouse and rat liver upon CS and THS (M) exposuresss.

CYP1A2 expression in the liver	CS			THS with HEETS (M)			CESS/SWITCH		
	Group[1]	FC[2] mRNA	FC[2] protein	Group[1]	FC[2] mRNA	FC[2] protein	Group[1]	FC[2] mRNA	FC[2] protein
ApoE$^{-/-}$ mouse study #1	3R4F 6m	1.39	**1.60***	THS 6m	1.05	0.96	CESS 6m	1.04	1.01
	3R4F 8m	1.44	**1.76***	THS 8m	1.11	1.00	CESS 8m	1.07	1.00
							SWITCH 6m	1.05	1.02
							SWITCH 8m	1.09	1.01
ApoE$^{-/-}$ mouse study #2	3R4F 6m	**1.35***	**1.38***	THS2.2 6m	1.06	1.05	CESS 6m	1.02	0.97
OECD 90-day rat THS2.2	3R4F-23-F-90d	1.11		THS2.2−23-F-90d	1.07				
	3R4F-23-M-90d	1.90		THS2.2−23-M-90d	1.25				
	3R4F-23-F-90d	1.37	1.33	THS M-23-F-90d	0.86	1.07			
	3R4F-23-M-90d	1.58	1.23	THS M-23-M-90d	1.19	1.12			
OECD 90-day rat THS2.2M	1XMIS-23-F-90d	1.44	1.38						
	1XMIS-23-M-90d	1.52	**1.30***						
	2XMIS-23-F-90d	**1.45***	**1.33***						
	2XMIS-23-M-90d	1.59	1.25						

Bold values represent statistically significant results.
Included studies: ApoE$^{-/-}$ mouse study #1 [24], ApoE$^{-/-}$ mouse study #2 [25], OECD 90-day rat THS [28], and OECD 90-day rat THS mentholated [26,27].
Group labels: Item–Time point (mouse studies), Item–Concentration (μg nicotine/L)–Sex (M (male), F (female))–Time point (rat studies), CESS, cessation, SWITCH, switching from 3R4F CS to THS aerosol.
Sample sizes: N = 8 (ApoE-/- mouse study #1, mRNA), N = 8 (ApoE-/- mouse study #1, protein), N = 9 (ApoE-/- mouse study #2, mRNA), N = 8 (ApoE-/- mouse study #2, protein), N = 6 (OECD 90-day rat THS2.2, mRNA), N = 6 (OECD 90-day rat THS2.2 M, mRNA), N = 6 (OECD 90-day rat THS2.2 M, protein).
[1] Group comparisons versus animals exposed to fresh air labeled as Item.
[2] Expression fold changes versus animals exposed to fresh air.
* False discovery rate-adjusted p value < 0.05. The false-discovery rate represents the proportion of false discoveries after adjustment for multiple-hypotheses testing of the evaluated omics data.

Table 3
CYP1A2 activity in smokers, switchers to THS, and abstainers in PMI's clinical studies (per protocol populations).

Study	Baseline			Day 5			Day 90		
	THS Mean (95 % CI)	CC Mean (95 % CI)	SA Mean (95 % CI)	THS Mean (95 % CI)	CC Mean (95 % CI)	SA Mean (95 % CI)	THS Mean (95 % CI)	CC Mean (95 % CI)	SA Mean (95 % CI)
5-day Poland (30)	112.4 (104.4, 120.4)	110.3 (100.7, 119.8)	113.1 (98.4, 127.9)	91.7 (85.2, 98.2)	123.0 (112.1, 134.0)	94.5 (82.6, 106.3)	NA	NA	NA
5-day Japan (29)	81.3 (73.9, 88.8)	78.2 (69.8, 86.6)	77.6 (69.6, 85.6)	56.6 (52.3, 60.8)	76.5 (68.7, 84.3)	52.3 (47.4, 57.1)	NA	NA	NA
90-day Japan (31)	76.0 (70.5, 81.4)	73.3 (66.6, 80.0)	78.5 (70.3, 86.7)	58.0 (54.0, 62.0)	79.8 (72.0, 87.7)	58.5 (52.5, 64.4)	57.6 (53.2, 62.0)	84.0 (73.6, 94.4)	64.0 (55.6, 72.4)
90-day U.S. (32)	123.6 (114.4, 132.8)	127.2 (113.9, 140.4)	121.7 (102.7, 140.6)	82.0 (75.0, 89.0)	127.9 (116.2, 139.6)	80.4 (65.6, 95.3)	78.9 (68.4, 89.4)	103.4 (87.9, 118.9)	99.9 (47.3, 118.9)

Table 4
Mean percent change from baseline CYP1A2 activity in smokers, switchers to THS, and abstainers in PMI's clinical studies (per protocol populations).

Study	Day 5			Day 90		
	THS % change (95 % CI)	CC % change (95 % CI)	SA % change (95 % CI)	THS % change (95 % CI)	CC % change (95 % CI)	SA % change (95 % CI)
5-day Poland (30)	−16.51 (-20.536, -12.481)	12.71 (7.705, 17.724)	−14.86 (-20.053, -9.667)	NA	NA	NA
5-day Japan (29)	−27.36 (-30.507, -24.216)	−0.99 (-5.964, 3.985)	−30.46 (-35.314, -25.609)	NA	NA	NA
90-day Japan (31)	−21.75 (-25.24, -18.26)	9.65 (2.43, 16.86)	−23.81 (-29.21, -18.41)	−20.22 (-26.66, -13.78)	15.77 (3.24, -28.30)	−15.82 (-23.19, -8.46)
90-day U.S. (32)	−32.81 (-36.702, -28.923)	3.56 (-1.121, 8.233)	−34.53 (-39.631, -29.433)	−31.96 (-40.407, -23.503)	−16.71 (-23.868, -9.545)	−35.36 (-53.476, -17.243)

Table 5
Mean percent change from baseline urinary 3-OH-B[a]P levels adjusted for creatinine (fg/mgcreat) in smokers, switchers to THS, and abstainers in PMI's clinical studies (per protocol populations).

Study	Day 5			Day 90		
	THS % change (95 % CI)	CC % change (95 % CI)	SA % change (95 % CI)	THS % change (95 % CI)	CC % change (95 % CI)	SA % change (95 % CI)
5-day Poland (30)	−71.43 (-76.65, -66.21)	−8.92 (-17.00, -0.84)	−77.24 (-81.14, -73.34)	NA	NA	NA
5-day Japan (29)	−64.76 (-72.17, -57.35)	−2.75 (-13.51, 8.02)	−71.15 (-78.84, -63.46)	NA	NA	NA
90-day Japan (31)	−68.42 (-74.27, -62.56)	−3.79 (-13.70, 6.11)	−67.04 (-76.32, -57.76)	−49.2 (-62.68, -35.71)	21.49 (-1.00, 43.99)	−46.51 (-61.64, -31.37)
90-day U.S. (32)	−68.31 (-74.94, -61.67)	−8.68 (-18.44, 1.08)	−75.41 (-83.48, -67.34)	−50.86 (-62.25, -39.47)	18.37 (-13.68, 50.41)	−41.82 (-63.36, -20.29)

There were two five-day studies and two 90-day studies with five-day confinement. The five-day periods of exposure in confinement was chosen to allow sufficient time for most biomarkers of exposure to tobacco smoke toxicants to reach steady state levels with THS 2.2 and SA arms (four to five times the half-life was estimated to lead to less than 5% of the original exposure levels of the assessed biomarkers on day five).

3.3. Five-day study in the EU (Poland)

Baseline CYP1A2 activity was comparable across the study arms (range, 110.3–113.1 %). At day five, the CYP1A2 activity in the THS and SA arms had decreased from baseline by approximately 17 % and 15 %, respectively, while that in the CC arm had increased by approximately 13 % [30]. At day five, the levels of total 3-OH-B[*a*]P had decreased from baseline by approximately 71 % and 77 % in the THS and SA arms, respectively.

3.4. Five-day study in Japan

Baseline CYP1A2 activity was comparable across the study arms (range, 77.6–81.3 %). At day 5, the CYP1A2 activity in the THS and SA arms had decreased from baseline by approximately 27 % and 30 %, respectively, while that in the CC arm had not changed significantly from baseline (an approximately 1% decrease) [29]. At day five, the levels of total 3-OH-B[*a*]P had decreased from baseline by approximately 65 % and 71 % in the THS and SA arms, respectively.

3.5. 90-day study in Japan

Baseline CYP1A2 activity was comparable across the study arms (range, 73.3–78.5 %). At day five, the CYP1A2 activity in the THS menthol and SA arms had decreased from baseline by approximately 22 % and 24 %, respectively, while that in the mCC arm had increased by approximately 10 %. At day five, the levels of total 3-OH-B[*a*]P had decreased from baseline by approximately 68 % and 67 % in the THS and SA arms, respectively. The CYP1A2 activity during the ambulatory period remained comparable to that seen on day five, with an approximately 20 % and 16 % decrease from baseline in the THS menthol and SA arms, respectively, and an increase of approximately 15 % from baseline in the mCC arm on day 90 [31]. The levels of total 3-OH-B[*a*]P at day 90 were still lower than the baseline levels by approximately 49 % and 46 % in the THS and SA arms, respectively.

3.6. 90-day study in the USA

Baseline CYP1A2 activity was comparable across the study arms (range, 121.7–127.2). At day five, the CYP1A2 activity in the THS

menthol and SA arms had decreased from baseline by approximately 34 % and 32 %, respectively, while that in the mCC arm had not changed significantly from baseline (an approximately 3% increase on day five). The CYP1A2 activity during the ambulatory period remained low, with an approximately 32 % and 19 % decrease from baseline in the THS menthol and SA arms, respectively. Subjects in the mCC arm also showed a decrease in CYP1A2 activity, with an approximately 16 % increase from baseline [32]. The levels of total 3-OH-B[*a*]P at day 90 were lower than those at baseline by approximately 51 % and 42 % in the THS and SA arms, respectively.

4. Discussion

The present article summarizes the evidence on the impact of switching to THS on 3-OH-B[*a*]P levels as a measurement of B[*a*]P exposure and CYP1A2 expression and activity. The preclinical and clinical studies assessed CYP1A2 expression and activity after switching to THS and after cessation of CS. The studies showed comparable reductions in CYP1A2 after switching (to THS exposure) and smoking cessation. Data from the clinical studies also showed a significant decrease in exposure to B[*a*]P, one of the PAHs present in tobacco smoke, both in the switchers to THS and in the SA groups. The levels of total 1-hydroxypyrene, a surrogate marker often used to assess exposure to PAHs, were also decreased in the switchers, comparably to the SA groups (data not shown) ([42]). As PAHs have been shown to induce CYP1A2 activity [2], having a reduced exposure to these compounds in particular explain the reductions of CYP1A2 activity observed in the smokers who switched to THS.

The evidence that cigarette smoking is a major modifiable risk factor for both pulmonary and cardiovascular disease is substantial [43]. However, despite the long-term reduction in morbidity and mortality associated with smoking cessation [43], quitting can also be associated with undesirable short-term physiological effects that are less well-known [44]. These potential adverse effects from quitting may hinder quit attempts if not actively managed or given special consideration [44]. Awareness and effective management of these negative effects by primary care physicians can result in better motivation and support for smokers, leading to more successful quit attempts. This is because cigarette smoking induces the expression of CYP1A2 enzymes, due to the exposure to the PAHs present in CS [45]. On the other hand, smoking cessation (or switching to THS) normalizes CYP1A2 enzyme levels [46].

4.1. Evidence for dose adjustment after smoking cessation or switching to electronic cigarettes (EC)

Smoking, with a consumption of 10–20 cigarettes per day, appears to be an inducer of CYP1A2, and the effect on drug metabolism will depend on the contribution ratio values for CYP1A2 (CR_{CYP1A2}) of the substrate drug. A study by MacLeod et al. [46], for instance, evaluated the results of caffeine phenotyping experiments to measure the effects of cigarette smoking on CYP1A2 activity. The authors reported that subjects in the smoking cessation group had a mean CYP1A2 activity of 17.8 (expressed as the urinary molar ratio of [17X + 17U] / 137X) while smoking; however, this activity decreased to 10.9 three weeks after cessation of smoking. Furthermore, a study by Faber and Fuhr [10] showed that after sudden smoking cessation (in heavy smokers), the initial caffeine clearance (estimated geometric means and 95 % confidence intervals) decreased significantly ($P < 0.01$) by 36.1 % (30.9–42.2 %) — from 2.47 mL/min $^{-1}$/kg^{-1} body weight (2.03–3.00 mL/min^{-1}/kg^{-1} body weight) to a new steady state of 1.53 mL/min^{-1}/kg^{-1} body weight (1.24–1.89 mL/min^{-1}/kg^{-1} body weight). The authors reported an apparent half-life of CYP1A2 activity decrease to be 38.6 h (27.4–54.4 hours), which is in line with our data showing reductions within a frame of five days [10].

Because of these findings, drugs that are primarily metabolized by CYP1A2 will have faster systemic clearance as a result of enzyme induction in smokers [2], while smoking cessation will reverse induced hepatic enzyme levels to normal [46] as well as reversing other smoking-induced effects, leading for instance, to the marked augmentation of plasma drug concentrations in patients whose dose was established while they were smokers [10]. Nicotine replacement treatment to assist smoking cessation will not influence this effect because, as previously mentioned, the effect on hepatic microsomal enzymes is not related to the nicotine component of smoking [47]. Determining the extent to which smoking cessation or switching to THS could alter the pharmacokinetics of existing drug regimens in a former smoker is, therefore, necessary for those individuals who want to quit and are currently receiving CYP1A2-metabolized medications [2].

The time course of CYP1A2 downregulation upon smoking cessation reported by both Macleod et al. [46] and Faber & Fuhr [10] is in line with the appearance of adverse events described in various case reports [2]. For instance, Bondolfi et al. [9] reported two cases of clozapine intoxication where, in the first patient, smoking cessation resulted in severe sedation and fatigue within two weeks, with an approximately 3-fold increase in plasma clozapine concentrations; the second patient presented very high plasma concentrations of clozapine (3004 ng/mL) six days after a 16-day stay in a general hospital, during which smoking was prohibited. Zullino et al. [17] reported two cases of patients who smoked cigarettes and cannabis. The first patient, who was receiving clozapine treatment, developed confusion after tobacco and cannabis cessation, which was related to an increase in plasma clozapine levels. The second patient, who was receiving olanzapine treatment, showed important extrapyramidal motor symptoms after reducing his cigarette consumption. Juergens [48] reported a case of a woman being treated with ropinirole for restless leg syndrome, who developed significant adverse events, including profuse sweating at night, four days after quitting smoking. Oyewumi [14] reported a case of a 46-year-old patient who was treated with clozapine and who, upon smoking cessation, complained of reappearance of urinary hesitancy, constipation, and erectile and ejaculatory dysfunction; the symptoms subsided after the clozapine dosage was reduced. McCarthy [13] reported a case of a 25-year-old man treated with clozapine and fluoxetine, who developed seizure following smoking cessation; the author reported that clozapine is associated with a dose-related risk of seizure, particularly in doses exceeding 600 mg/day. Similar cases of clozapine toxicity after smoking cessation have been reported by Skogh et al. [15]. Finally, a study by Meyer [16] on 11 patients receiving treatment with clozapine found that, after abrupt smoking cessation, there was a mean increase of 71.9 % (442.4 ± 598.8 ng/mL in clozapine levels from a baseline level of 550.2 ± 160.18 ng/mL; $p < 0.034$).

Similar results have been reported after switching from smoking to EC use. ECs are inhalation devices that deliver nicotine without tobacco smoke [49]. These products are free of combustion-related toxicants, including PAHs; therefore, unlike cigarettes, they do not interfere with drug metabolism [50]. As expected, possible adverse effects may occur if a patient transitions from cigarette smoking to ECs without a dose adjustment [51]. At least three reports of adverse events due to switching from smoking to EC use in patients prescribed with clozapine have been published. The first case, reported by Khorassani et al. [51], refers to a 52-year-old man who switched to ECs and experienced a substantial increase in clozapine concentration, causing the patient to become symptomatic. The second report by Kocar & Freudenmann [52] described a 23-year-old female patient medicated with 300–550 mg/day of clozapine. With the higher dose, the patient reported symptoms of clozapine overdosing, which was due to the patient switching from smoking 10–15 cigarettes a day to using ECs (with nicotine); the symptoms subsided upon reducing the dosage. The last report was from Berm et al. [53], who presented a case of a 34-year-old patient with paranoid schizophrenia being treated with clozapine; the patient experienced drowsiness after reducing cigarette consumption and switching to EC use. The symptoms were resolved by halving the dose of clozapine.

4.2. Recommendations after smoking cessation and switching to THS or other RRPs

There is substantial evidence showing that smoking cessation results in downregulation of CYP1A2 expression [2]. Case reports of smokers switching to EC use have shown similar findings [51,52]. Additionally, the present article shows that switching to the THS will reduce CYP1A2 activity. Because the CYP1A2 activity seen in those who switch to THS is comparable to that seen upon smoking cessation, these conclusions could be extrapolated to switching to the THS (or other RRPs that guarantee reduced exposure to PAHs). Smoking cessation reverses induced hepatic enzyme levels [46] and also reverses other smoking-induced effects [10]. Increased plasma concentrations of CYP1A2-metabolized drugs after smoking cessation may cause important clinical consequences, particularly in the case of drugs with narrow therapeutic indices [54], including warfarin, clozapine, olanzapine, and theophylline [10,55].

Due to the short turnover time of CYP1A2, empirical dose reduction may be necessary within two to three days after smoking cessation [2,10] or switching to RRPs, although there are currently no recommendations or guidelines on dose adaptation of CYP1A2-metabolized drugs upon smoking cessation. In patients taking drugs with narrow therapeutics indices, close monitoring of clinical symptoms for adverse events is necessary within the first week of smoking cessation (or switching to RRPs) [56]. This issue needs to be addressed particularly in smokers with psychiatric comorbidities who use several types of concomitant medications [44].

This publication summarizes data from preclinical and clinical studies on CYP1A2 expression and activity after exposure to THS. The results presented here pertain to primary analyses, and the study populations included in the clinical studies were composed of different ethnicities. The minimum study length (five days) was optimal for allowing that most biomarkers of exposure to tobacco smoke toxicants to reach steady state levels with THS and smoking abstinence arms. Nevertheless, there are some limitations in this approach. Firstly, there is the possibility of adaptation and compensation among new users of THS. In this aspect, the studies conducted thus far have shown varying patterns of product consumption during the first days of exposure to the new product with different characteristics compared to subjects' own brand of cigarettes. This was expected and part of the adaptation process to a new product such as THS. These variations in product consumption observed early on after switching to THS tended to disappear over time, as assessed by measuring urinary levels of nicotine equivalents and self-reported tobacco products, which indicates that there is little or no compensation after the adaptation period. Secondly, we did not include in our review results from independent studies assessing CYP1A2 activity and expression in those switching to THS. This was due to a lack of such studies in the literature.

5. Conclusions

Both switching to RRPs and smoking cessation could potentially lead to elevations of CYP1A2-metabolized drug concentrations. CYP1A2 is involved in the metabolism of many commonly used drugs [57]. Because of the aforementioned findings, patients who switch to RRPs and are taking drugs that are substrates for CYP1A2 should communicate this to their treating physicians in order for them to consider a dose modification. Additionally, physicians should be aware of the potential adverse effects associated with smoking cessation, which might affect a smoker's attempt to quit [44], as well as the potential effects of switching to RRPs.

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CRediT authorship contribution statement

Angela van der Plas: Conceptualization, Methodology, Investigation, Project administration. **Sandrine Pouly:** Data curation, Resources, Formal analysis. **Nicolas Blanc:** Conceptualization, Methodology, Supervision. **Christelle Haziza:** Methodology, Resources. **Guillaume de La Bourdonnaye:** Data curation, Formal analysis. **Bjorn Titz:** Formal analysis, Data curation. **Julia Hoeng:** Methodology, Resources. **Nikolai V. Ivanov:** Methodology, Resources. **Brindusa Taranu:** Data curation. **Annie Heremans:** Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.10.017.

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