禁煙推進はライフワーク クリニック経営は在宅でカバー

薗はじめクリニック　院長　**薗　はじめ**

大阪府豊中市にある「薗はじめクリニック」は、禁煙外来と在宅医療に特化するという非常に珍しいクリニックだ。院長の薗はじめ氏は、熱心な禁煙推進活動家としての顔も持つ。年間50回ほどの講演会をこなすとともに、世界禁煙デーにはパレードを実施、在宅の患者、家族にも禁煙を約束してもらう徹底ぶりだ。「タバコを博物館の展示品にすることが目標」と語る薗氏に、これまでの取り組みについて話をうかがった。

禁煙外来は社会貢献の一環 経営の柱は在宅医療

――薗はじめクリニックでは、禁煙外来と在宅医療の２つに特化するという非常にユニークな体制を敷いておられますね。その経緯をお聞かせください。

薗　筑波大学医学部を卒業後、臨床でタバコの被害を目の当たりにしたことがきっかけです。“早死に”の原因のほとんどはタバコでした。喫煙者の２人に１人は喫煙が原因で死ぬという統計に接し、「禁煙支援を行わないのは不作為の罪」と強く感じ、青森県初の禁煙外来を開設しました。当時は今以上にタバコ問題の認識が不十分で、周囲の反応も的はずれでしたが、各地で禁煙推進に取り組む仲間と連絡を取り合い元気をもらいました。タバコ対策は、私のライフワークと決め、赴任先の病院で次々と禁煙外来を開設しました。

一方、私が赴任した青森の精神科の病院では患者さんの社会復帰に、内科の病院でも在宅医療に先駆的に力を入れていました。どちらも、その人らしい生活を支える医療です。

「禁煙外来と在宅にもっと専念したい」と考えていた矢先、禁煙運動で知り合った夫との結婚をきっかけに関西に移り住んだ私は、3年間の病院勤務を経て、禁煙外来と在宅医療に特化したクリニックを2004年に開業しました。

――現在、どれくらいの患者さんを診ているのですか。

薗　禁煙外来は完全予約制で火曜と木曜が13～18時まで、月曜、水曜、金曜は17～18時が予約枠です。波はありますが毎週１～２人の新患さんが来られます。在宅の患者数は約80人です。非常勤医師もおり、１日９件程度の患者宅を回ります。平日の午前と禁煙外来のない午後に行っています。

――経営的に考えると、禁煙外来だけで採算を合わせるのは困難です。同時に一般外来に取り組むという選択もあったと思います。

薗　「ニコチン依存管理料」では採算はとれません。１人ひとりの診療時間を十分にとらなければ、適切な治療はできませんので、一度に多くの患者さんは診られません。禁煙外来は初診で1時間以上、再診でも15分以上かかります。

禁煙啓発の講演の時間を確保するために、完全予約制で予定が立てやすいのも利点です。

当院の経営の柱は在宅です。禁煙外来は10年以上も、低額の自由診療によるボランティアのような時代を経て、ようやく保険診療が可能になりました。今でも禁煙外来は採算が取れず、社会貢献の一環として取り組んでいるような側面があります。

薬物療法とカウンセリングで ９割が１か月以上の禁煙に成功

――禁煙指導では特にどのようなことを重視しておられますか。

薗　まず、喫煙はニコチン依存症という病気であることを自覚していただくことが大切です。

禁煙は意志が弱いからできないわけではありません。脳に対し、ニコチンという薬物が作用しているために止められないのです。意志が弱いなどと自分を責める必要はありません。

吐く息の一酸化炭素濃度や尿のニコチン代謝物濃度を検査して、科学的にデータを示すことは、タバコの被害を認識し、禁煙を考えるきっかけにもなります。また禁煙の効果を知るよい指標となります。

――そこから、カウンセリングや治療に進んでいくわけですね。

薗　カウンセリングは**「５つのA」**(下欄参照)と呼ばれるアプローチ方法に沿って進めます。

喫煙開始時期、喫煙本数、職場や家庭の喫煙環境など、現在の喫煙状況をよく聞き、リスクをはっきり伝えます。禁煙への心構えを評価し、禁煙を妨げる要因に前もって注意しながら具体的な禁煙計画を立てていきます。

治療は、飲む禁煙薬「チャンピックス」や、パッチやガムなどのニコチン製剤をケースに応じて併用します。あとは吸わない生活に慣れていく練習も重要です。

――禁煙する気持ちが弱い人に対する指導は難しいでしょうね。

薗　喫煙者はたいてい禁煙のきっかけを求めているものですから、適切なアプローチにより、禁煙に踏み出せます。そこでは**「5つのR」**と呼ばれるアプローチ方法が役立ちます。

特に重要なのは「Relevance:関連性」。患者さんにタバコの被害が他人事ではなく、自分に関連していることに気付いていただきます。たとえば、喫煙によるがんや脳卒中の危険性には無関心な若者も勃起不全や美容への悪影響を知ることで禁煙に興味を持つことがあります。挙児希望のカップルでは死んでも止めないと言った妻が夫の分まで禁煙のパンフレットを持って帰った事例がありました。自分が大切だと思うことを妨げる要因としてタバコを自覚できた時、決心のスイッチが入ることがあります。自分にとって大切と思うことは人それぞれ異なりますので、医療者はタバコ問題に対し、たくさんの引き出しを用意しておくことが必要です。

――指導の結果はいかがですか。

薗　ニコチン依存管理料は５回の外来診療が必須で、中断を防ぐことが重要です。当院では、終了まで通う患者さんは８割、その９割が１か月以上の禁煙に成功しています。

受動喫煙症の診断を行い タバコの被害者をサポート

――禁煙に関する講演を数多く行うなど、啓発活動にも力を入れておられますが。

薗　毎年各地で講演会を行うとともに、5月31日の世界禁煙デーには兵庫県喫煙問題研究会の活動を通じて、禁煙パレードを実施しています。毎年のように国際学会に出席し、タバコ対策のグローバルスタンダードや禁煙指導のあり方を学んでいます。

またマイカーの車体には大きな「SMOKING KILLS」の文字と禁煙マークを掲げています。往診車

●参考：禁煙指導における「５つのA」と「５つのR」

すべての喫煙者へのアプローチ「５つのA」

1. **Ask**(問診)
   喫煙状況を聞く。
2. **Advice**(忠告)
   止めるようにきっぱり言う。タバコの危険性を明確に示す。
3. **Assess**(評価)
   止める気があるかどうかを確認する。なければ「５つのR」へ。
4. **Assist**(支援)
   止める日を決める。
5. **Arrange**(手配)
   予定を立てる。次の来院日を決める。

タバコを止める気がない喫煙者に対する「５つのR」

1. **Relevance**(関連性)
   患者さんに関連がある情報を提供し、自分の身にも起き得ることだと気付かせる。
2. **Risks**(リスク)
   健康被害が何より危険だと知らせる。
3. **Rewards**(報酬)
   多くのよいことがあることを知らせる。
4. **Roadblocks**(障害)
   禁煙の成功を妨げる要因を明らかにし、禁煙の障害を乗り越える方法を伝える。
5. **Repetition**(反復)
   失敗を恐れず何度も挑戦することを伝える。

にも禁煙マークとともに、「禁煙指導保険診療」の文字があります。禁煙治療に保険が適用することを知っている人は3～4割に過ぎないと指摘されているなかで、この文字を見て、禁煙を決意した人もいました。

――タバコをなくしたいという強い思いが伝わってきますね。

薗　日本では毎年11万3,000人の喫煙者がタバコで命を落としています。非喫煙者も、2～3万人が受動喫煙で毎年亡くなっています。タバコを1日も早く非合法化しなければなりません。

日本はタバコをなくすために、世界169か国が批准する「タバコ規制枠組み条約」を2004年6月に批准しています。そこには2010年2月までに屋内を例外なく全面禁煙にする法律を罰則規定付きで実施することが盛り込まれましたが、日本では、期日が過ぎても、厚生労働省からアリバイ的な通知が出ただけです。

また、最近まとめられた職場の受動喫煙対策に関する検討会報告書では、「やむを得ず禁煙にできない場合は喫煙室を設置する」旨の文言が入りました。やむを得ず禁煙にできないケースなど、諸外国を見てもどこにもありません。「マスクをして被害を軽減する」旨の内容も入っています。受動喫煙がマスクで軽減できるというエビデンスはありません。そもそも、なぜ非喫煙者がマスクをしなければならないのか。検討会でこういう報告書がまとめられるのは許されることではありません。

――日本はもっと真剣に受動喫煙対策に取り組むべきですね。

薗　そのために私たち1人ひとりが声を上げ、行動していかなければならないと啓発活動を通じて呼びかけています。

当院では受動喫煙被害者の方々の禁煙化要望や、訴訟対策として、受動喫煙症の診断も行っています。訴訟件数が増えれば社会は変わりますが、被害者が打ちのめされていて、訴訟までつながらないのが現実です。今後もサポートを続けていきたいです。

認知症の在宅においても禁煙支援に取り組む

――先生のそうした活動の原動力はどこにあるのでしょうか。

薗　タバコとの戦いは命と健康を守る医療従事者として、当然の使命です。「その人がその人らしく人生を全うすることを支えたい」という思いは在宅にもつながります。当院の在宅は認知症の患者さんが中心です。内科的には安定した方が多いのですが、在宅療養支援診療所として、24時間365日体制も整えており、他の医療機関との連携も密に行っています。

ご自宅で病気と付き合うことは、患者さんもご家族も、不安なものです。その不安なお気持ちを先回りして解消することで、突発的な問題をかなり減らすことができます。住み慣れたところで生き生きと暮らす方々を訪問するのは楽しい仕事です。

また在宅でも禁煙は治療の大前提です。患者さんや家族に喫煙者がいた場合、必ず禁煙していただいています。同意いただけない場合は在宅診療をお断りします。感染症対策同様、訪問する私たちの受動喫煙被害を防ぐ必要がありますし、喫煙していてはあらゆる治療は意味がなくなるからです。

ニコチンという薬物に支配されている状態はその人本来の姿ではありません。当院との関わりがきっかけで禁煙に成功した患者さんやご家族がたくさんおられ、とても喜んでくださっています。

――これからもその人らしい生き方を支えるために在宅、そして、禁煙活動に積極的に取り組んでいくのでしょうね。

薗　高齢者の増加にともない、認知症の在宅ニーズに適切に応えられるように、さらなる体制整備、連携強化に努めていきたいです。

タバコ対策の最終的な目標は1日も早くタバコを博物館の展示物にすることです。そのために医療者としてできることは何でもやっていきたい。「昔はタバコでたくさんの人が亡くなったけど、今はなくなってよかった」と言える社会がくるまで、全力でがんばりたいと思っています。

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Short Communication

Cigarette Smoke Extract Induces CYP2B6 through Constitutive Androstane Receptor in Hepatocytes[S]

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ABSTRACT:

Smoking induces a wide range of drug-metabolizing enzymes. Among them, CYP2B6 as well as CYP1A2 is well known to be up-regulated in smokers. Although the induction of CYP1A2 is mediated by the aryl hydrocarbon receptor, the molecular mechanisms of CYP2B6 induction by smoking remain to be fully elucidated. In this study, by preparing cigarette smoke extract (CSE), we addressed the possibility that human constitutive androstane receptor (hCAR) is involved in smoking-mediated induction of CYP2B6. In HepG2 cells, CSE induced CYP1A2 but not CYP2B6, suggesting that CYP2B6 expression is differentially regulated from CYP1A2. Compared with liver in vivo, hCAR expression is dramatically reduced in cultured hepatocytes, such as HepG2. Therefore, to reconstitute hCAR signaling pathways in vitro, we generated adenovirus vector expressing hCAR. Real-time reverse transcription-polymerase chain reaction analyses revealed that the adenoviral transfection of hCAR resulted in the up-regulation of CYP2B6 mRNA, even in the absence of CSE. It is interesting to note that CSE stimulation augmented hCAR-mediated induction of CYP2B6. In contrast, the expression of CYP2B6 was not enhanced by adenovirus vector expressing β-galactosidase, a control vector, either in the presence or absence of CSE. In summary, hCAR mediated the CYP2B6 induction by CSE in Hep2G cells. These data suggest that smoking up-regulates CYP2B6 through hCAR in vivo.

Introduction

In addition to CYP2A6, CYP2B6 is one of the major nicotine-metabolizing enzymes (Yamazaki et al., 1999). Much attention has been paid to the regulation of CYP2B6 activity, because nicotine-metabolizing activity is positively associated with nicotine dependence, and the understanding of nicotine dependence contributes to the promotion of smoking cessation (Ray et al., 2009). So far, several *CYP2B6* genetic polymorphic mutations that affect CYP2B6 enzyme activity have been identified; however, the association of *CYP2B6* genotypes with nicotine dependence is still controversial (Lee et al., 2007; Ring et al., 2007). It is interesting to note that CYP2B6 expression is up-regulated in smokers (Miksys et al., 2003), suggesting that cigarette smoke induces CYP2B6. Therefore, the understanding of molecular mechanisms for CYP2B6 induction by smoking would provide a novel insight into nicotine dependence.

Smoking induces drug-metabolizing enzymes, including CYP1A2 and CYP2B6, and influences the pharmacokinetic profiles of a wide range of drugs (Kroon, 2007). It has been established that cigarette smoke activates aryl hydrocarbon receptor (AhR) and up-regulates CYP1A2 (Shimada et al., 2002), resulting in the increased clearance of CYP1A2 substrates, such as theophylline. In contrast to CYP1A2, the involvement of AhR in CYP2B6 expression has not been proved. It has been demonstrated previously that *CYP2B6* gene expression is regulated by human pregnane X receptor and human constitutive androstane receptor (hCAR) (Wang and Negishi, 2003). *CYP2B6* gene is the target of hCAR, and the direct binding sites for hCAR have been identified in the *CYP2B6* gene promoter region (Swales et al., 2004); however, whether hCAR is involved in CYP2B6 induction by cigarette smoke remains to be elucidated.

In this study, we have designed experiments to address the effects of tobacco smoke on CYP2B6 expression in vitro. In analysis on the molecular mechanisms of the expression of drug-metabolizing enzymes in vitro, the difficulty is derived from the phenotypic changes during the cell cultivation. In particular, it is well known that hCAR expression is down-regulated immediately after hepatocyte cultivation from liver (Pascussi et al., 2000). Therefore, hCAR signal is likely to be impaired in cultured hepatocytes. In this context, we have generated the adenovirus vector expressing hCAR (Ad-hCAR). By transducing the *hCAR* gene, we reconstituted hCAR signaling systems in cultured hepatocytes and analyzed the effects of cigarette smoke extract (CSE) on CYP2B6 induction in HepG2 cells. In addition, we have revealed that hCAR expression is required for the CYP2B6 induction by CSE in HepG2 cells but not for that of CYP1A2. These data suggest that smoking induces CYP2B6 through the CAR signaling pathway.

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ABBREVIATIONS: AhR, aryl hydrocarbon receptor; hCAR, human constitutive androstane receptor; Ad-hCAR, adenovirus vector expressing hCAR; CSE, cigarette smoke extract; PBS, phosphate-buffered saline; RT-PCR, reverse transcription polymerase chain reaction; HEK, human embryonic kidney; MOI, multiplicity of infection; Ad-βgal, adenovirus vector expressing β-galactosidase.

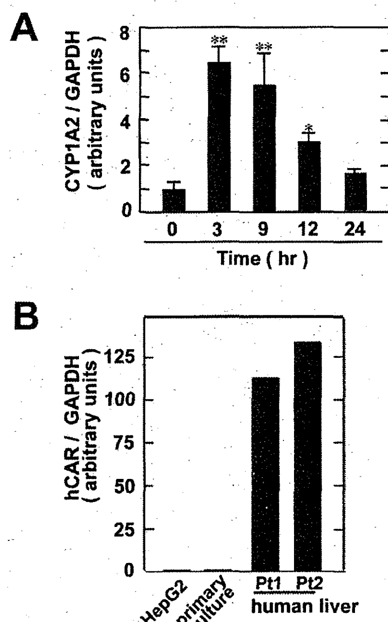

FIG. 1. A, CSE up-regulated the expression of CYP1A2 in HepG2 cells. HepG2 cells were cultured with 7% CSE for the indicated time. The expression of CYP1A2 was analyzed by real-time RT-PCR. The expression of the drug-metabolizing enzymes was normalized with that of glyceraldehydes-3-phosphate dehydrogenase (GAPDH). Data are shown as means ± S.D. from 4 samples. **, $p < 0.01$ versus 0 h; *, $p < 0.05$ versus 0 h. Statistical analyses were performed by one-way analysis of variance followed by post hoc multiple comparisons with the Tukey-Kramer test. B, the expression of hCAR mRNA in HepG2 cells and human liver. Total RNA was prepared from HepG2 cells, primary hepatocytes, and human liver samples. The expression of hCAR mRNA was estimated by real-time RT-PCR. The expression level of hCAR was normalized by that in HepG2 cells.

Materials and Methods

CSE Preparation. CSE was prepared according to a method described previously (Su et al., 1998), with minor modifications. In brief, commercial cigarettes (Seven Stars; JT Group, Tokyo, Japan) were smoked continuously through phosphate-buffered saline (PBS) by the vacuum aspirator. Mainstream smoke from 10 cigarettes was drawn through 20 ml of PBS by application of a vacuum aspirator to the vessel containing the PBS, which was designated as 100% CSE. For each experiment, this solution was diluted with PBS to the indicated concentrations.

Generation of the Adenovirus Vector Expressing hCAR. The construction of the adenovirus vector was performed according to a method described previously (Becker et al., 1994). In brief, full-length human CAR cDNA was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using human liver cDNA as the template. The sequences of the primers were GGGGAATTCATGGCCAGTAGGGAAGATGAGCTGAGGAACTGTGTGG-TATGTGG (forward) and GGGAAGCTTTCAGCTGCAGATCTCCTGGA-GCAGCGGCATCATGGCAG (reverse). The PCR product was digested with EcoR1 and Hind3 and ligated into the multicloning site of pCCMVpLpA vector. The plasmid pACCMVpLpA-hCAR was cotransfected with pJM19 into HEK293 cells for homologous recombination.

Cell Culture. HepG2 cells were maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum.

In adenoviral infection, cells were cultured overnight with adenovirus vectors at the indicated multiplicity of infection (MOI). Culture media were changed to Eagle's minimum essential medium with 10% fetal bovine serum (CELLect Gold; MB Biomedicals, Inc., Aurora, Ohio) without adenovirus particles. Twenty-four hours later, cells were stimulated with the media containing CSE.

Real-Time RT-PCR Analysis. Real-time RT-PCR was performed as described previously (Mohri et al., 2009). Total RNA (2 μg) was subjected to first-strand cDNA synthesis with oligo(dT) primer. The expression of each template was quantified by real-time RT-PCR with a SYBR Green system (Applied Biosystems, Foster City, CA). As an internal control, the expression of glyceraldehydes-3-phosphate dehydrogenase mRNA was estimated with a SYBR Green system. The primer pairs used in this study were demonstrated in the supplemental table.

Immunoblot Analysis. Cell lysates were prepared by directly extracting proteins with an SDS-polyacrylamide gel electrophoresis sample solution. Proteins were separated with SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene membrane. After being blocked with 5% skim milk, the membrane was incubated with anti-hCAR (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). An enhanced chemiluminescence system (ECL Plus Western Blotting Detection Reagents; GE Healthcare Japan, Co., Ltd., Tokyo, Japan) was used for detection.

Results and Discussion

In this study, we have introduced CSE into the analysis on the expression of drug-metabolizing enzymes. CSE has been used mainly to address the effects of cigarette smoke on tissue damage, such as endothelial dysfunction (Hoshino et al., 2005). To our knowledge, this is the first application of CSE to address the influences of cigarette smoke on drug-metabolizing enzymes. Therefore, first we examined the effects of CSE on CYP1A2 expression in cultured hepatocytes (Fig. 1A) because CYP1A2 is well known to be induced by smoking through the activation of AhR. CYP1A2 mRNA was remarkably increased within 3 h after CSE stimulation, indicating that CSE could be a good tool for the study of drug metabolism. In contrast to CYP1A2, CYP2B6 mRNA was not induced by CSE in HepG2 cells (data not shown).

Because hCAR is one of the transcriptional factors responsible for CYP2B6 expression, the expression of hCAR in HepG2 was examined. Real-time RT-PCR estimated that the expression level of hCAR in HepG2 cells is less than 1% of that in human liver samples (Fig. 1B), consistent with the previous report that hCAR is rapidly down-regulated after cultured hepatocyte preparation from liver (Pascussi et al., 2000). Therefore, to reconstitute the hCAR signaling pathway in HepG2 cells, Ad-hCAR was generated (Fig. 2A). To confirm the expression of hCAR protein, HepG2 cells were infected with Ad-hCAR or adenovirus vector expressing β-galactosidase (Ad-βgal), a control vector. Cell lysates were prepared and immunoblotted with anti-hCAR antibody (Fig. 2B). The band with a molecular mass of 40 kDa was detected in Ad-hCAR-infected HepG2 cells, as reported previously (Arnold et al., 2004), although not in Ad-βgal-infected cells.

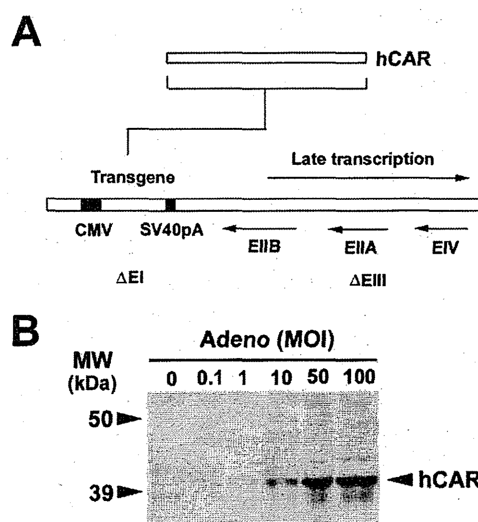

FIG. 2. Generation of adenoviral vector expressing hCAR. A, construction of adenovirus vector expressing hCAR (Ad-hCAR). CMV, cytomegalovirus. B, HepG2 cells were infected with Ad-hCAR at the indicated MOI. Cell lysates were prepared and immunoblotted with anti-hCAR antibody (Santa Cruz Biotechnology, Inc.).

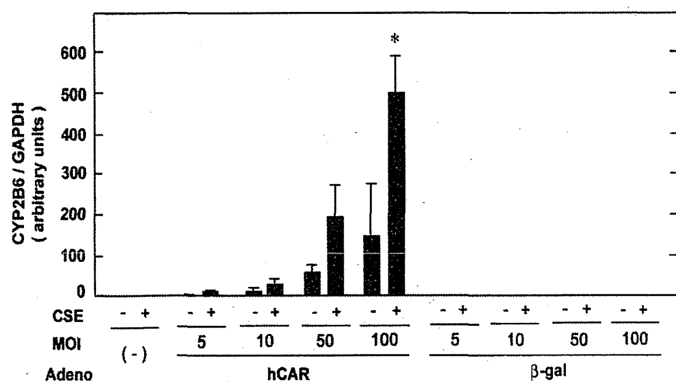

FIG. 3. CSE enhanced CYP2B6 expression in HepG2 cells infected with Ad-hCAR but not in those infected with Ad-βgal. HepG2 cells were infected with Ad-hCAR and Ad-βgal at the indicated MOI. Cells were stimulated with 7% CSE for 24 h. The expression of CYP2B6 was analyzed by real-time RT-PCR. Data are shown as means ± S.D. from 3 independent sets of samples. *, $p < 0.05$ versus CSE(−), analyzed by paired t test.

To examine whether hCAR mediates CYP2B6 induction in response to CSE, HepG2 cells were infected with Ad-hCAR or Ad-βgal at the indicated MOI and stimulated with CSE (Fig. 3). At a high MOI, adenoviral transfer of hCAR cDNA up-regulated CYP2B6 mRNA, even in the absence of CSE, as reported previously. It is important to note that CYP2B6 expression was dramatically enhanced by CSE in Ad-hCAR-infected HepG2 cells, but not HepG2 cells infected with Ad-βgal, a control vector. We used real-time RT-PCR to confirm that CSE increased the expression of CYP2B6 mRNA in HepG2 cells expressing hCAR in a concentration-dependent manner (Supplemental Fig. 1). It is interesting to note that the damage in HepG2 cells expressing hCAR was observed after stimulation with 8 to 10% CSE. Therefore, 7% CSE was used to stimulate the cells for this study. The most important finding of this study is that CSE enhanced CYP2B6 expression in cultured hepatocytes expressing hCAR, indicating that CSE-dependent expression of CYP2B6 is mediated by hCAR. Because the CSE increased CYP1A2 expression, either in Ad-hCAR- or Ad-βgal-infected HepG2 cells (data not shown), CSE differentially regulates the gene transcription of *CYP2B6* from that of *CYP1A2*.

This is the first demonstration that cigarette smoke contains the activator of hCAR transcription factor and regulates the transcription of drug-metabolizing enzymes. Although smoking influences the expression for drug-metabolizing enzymes, it has been difficult to address its regulatory mechanisms of the enzyme expression without the identification of the components that activate the transcription factors for the enzymes. In this study, we have successfully addressed the effects of cigarette smoke on the expression for CYP2B6 by using CSE and Ad-CAR. The in vitro system used in this study might be a promising approach for smoking-drug interaction.

In conclusion, we have established a novel method that is available for molecular analysis of the induction of drug-metabolizing enzymes by smoking. In addition, by using this system, we have revealed that cigarette smoke induces CYP2B6 through hCAR in HepG2 cells, proposing the molecular mechanisms for CYP2B6 induction by smoking in vivo.

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