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Constitutive Expression and Changes of Cytochrome P450 Isozymes mRNAs by Vehicles (Petrolatum, DMSO, Ethanol) in Rat Skin Using Semi-quantitative RT-PCR

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Many drugs are primarily metabolized by the cytochrome P450s (CYPs). Drug metabolites would be important allergens for adverse drug reactions such as drug eruptions. Skin tests with a suspected drug have conducted to identify causative drugs of drug eruptions, with vehicles such as white petrolatum, DMSO, ethanol. This study will compare the expression of rat CYP isozyme mRNAs between the skin and the liver, with examining an effect of the vehicles on the cutaneous CYPs using semi-quantitative RT-PCR. Thirty-two Sprague-Dawley rats between the ages of six and eight weeks were divided as four groups. One group was used to compare the constitutive mRNA expression between skin and liver, while the others were to examine the effects of three vehicles. The ratios of expression of CYP1A2, CYP2B1/2, CYP2E1, CYP3A1, and CYP4A1 were significantly higher in the liver than the skin. However, CYP1A1 and CYP2C11 were higher in the skin than liver. The effects of vehicles were quite different; white petrolatum significantly induced CYP1A1 ($p=0.012$) and CYP2C11 mRNAs, while ethanol inhibited CY P1A1 and CYP2B1/2. DMSO did not make any changes. The results suggest that rat skin can participate in drug metabolism with their own CYP isozymes. The effects of vehicles on the cutaneous CYP expression should not be ignored and may be applied for determination of an appropriate vehicle for certain drug(s).

Key Words: Cytochrome P450 (CYP) mRNAs, Liver, Skin, White petrolatum, DMSO, Ethanol

INTRODUCTION

Many drugs are primarily metabolized by the cytochrome P450s (CYPs). The metabolites generated by the CYPs are pharmacologically active and can be conjugated with endogenous substrates. Conjugation to macromolecules is necessary to eliminate drugs from the body and also contributed to be an allergen by overcoming their small molecular weight (Merk et al, 1997). The latter suggests an important role of CYPs in pathogenesis of drug eruption, which is considered to be an allergic mechanism.

Drug metabolism by the CYPs is difficult to study because of many CYPs members and their diverse substrate array. CYPs have been usually examined in the liver. The study of CYPs in the skin is necessary to understand the cutaneous metabolic processes of certain drugs and their important metabolites. Although CYPs have interspecies difference, it is very difficult to compare the cutaneous and hepatic human CYPs in vivo. Animal skin would be a reliable substitute for human skin in the study of drug metabolism.

Topical provocation with suspected drugs has been used for identification of cause in drug eruptions.

White petrolatum, DMSO, or ethanol has been

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popularly used for the preparation of testing drugs as vehicles. Their effects have been examined *in vitro* (Busby et al, 1999), not *in vivo*.

This study examined the expression of CYP isozymes in rat skin and compare them with hepatic CYPs, with the change of cutaneous CYPs after topical application of white petrolatum, DMSO, or ethanol.

METHODS

Animal preparation

Male Sprague-Dawley rats with ages of 7~8 weeks and weighs of 200~300 g were used for this study. The total number was 32. Food and water were *ad lib*.

Rats were divided into four groups; without vehicles, with white petrolatum, with DMSO, and with ethanol. The number of an each group included eight rats. The eight rats cut back hair without applying any vehicles. A vehicle was occluded for two days on the lower back of each rat group after removing the hairs. The upper back of each rat was remained without applying vehicles for a control. Hair removal conducted after an intra-peritoneal injection of anesthetics, avertin (5%, 0.8 cc/100 g of body weight).

Primer preparation

The oligonucleotides from 8 kinds of CYP isozymes, CYP1A1 (331 bp), CYP1A2 (236 bp), CYP2B1/2 (549 bp), CYP2C11 (248 bp), CYP2E1 (473 bp), CYP3A1 (579 bp), and CYP4A1 (344 bp), which are reported as the major isozymes in the rat (3), were synthesized. CYC (cyclophyllin, 265 bp) was used as a house-keeping gene. The sequences of the primers were as follows (3): CYC sense primer 5'-CTTCG ACATCACGGCTGATGG-3', CYC anti-sense primer 5'-CAGGACCTGTATGCTTCAGG-3', CYP1A1 sense primer 5'-CTGGTTCTGGATACCCAGCTG-3', CYP1A1 anti-sense primer 5'-CCTAGGGTTG-GTTACCAGG-3', CYP1A2 sense primer 5'-GTCA-CCTCAGGGAATGCTGTG-3', CYP1A2 anti-sense primer 5'-GTTGACAATCTTCTCCTGAGG-3', CYP2B1/2 sense primer 5'-GAGTTCTTCTCTGGGTT-CCTG-3', CYP2B1/2 anti-sense primer 5'-ACTGTG-GGTCATGGAGAGCTG-3', CYP2C11 sense primer 5'-CTGCTGCTGCTGAAACACGTG-3', CYP2C11 anti-sense primer 5'-GGATGACAGCGATACTAT-CAC-3', CYP2E1 sense primer 5'-CTCCTCGTCA-TATCCATCTG-3', CYP2E1 anti-sense primer 5'-G-CAGCCAATCAGAAATGTGG-3', CYP3A1 sense primer 5'-ATCCGATATGGAGATCAC-3', CYP3A1 anti-sense primer 5'-GAAGAAGTCCTTGTCTGC-3', CYP4A1 sense primer 5'-GGTGACAAAGAAC-TACAGC-3', CYP4A1 anti-sense primer 5'-AGAG-GGTCTTGACCTGCCAGCYC-3'.

Total RNA preparation from skin and liver

Rats were killed by cervical dislocation. 1 cm^2- sized pieces of back skin and liver were cut from the rats without vehicles. Two pieces of back skin were removed from the rats with a vehicle. The removed pieces were immediately transferred to the tubes containing RNAlater® (Ambion, 7020) for protection of RNA degradation. The specimens ground after freezing with liquid nitrogen. Total RNA was extracted using Trizol® (GibcoBRL, 15596-026).

Reverse transcriptase-PCR (RT-PCR)

Total RNA was converted to cDNA using a 1st Strand cDNA Synthesis Kit for RT-PCR (AMV) (Boehringer Mannheim, 1483 188). PCR conducted with the primers, 8 isozymes and a house-keeping gene, in a DNA thermal cycler (Perkin-Elmer 9600) under the conditions of 94°C for 30 sec for degeneration, 56°C for 1 min for annealing, and 72°C for 1 min for extension. The amplifications were done for 23 cycles. The electrophorezed bands were photographed and measured by a densitometry (Pharmacia).

Statistical evaluation

The results were analyzed by a wilcoxon signed ranks test.

RESULTS

CYP isozymes mRNA expression between skin and liver (Fig. 1A, 1B).

All CYP isoenzymes were expressed constitutively in the liver. Constitutive CYP isozymes in rat skin were different from those in the liver. The levels of CYP1A2, CYP2B1/2, CYP2E1, CYP3A1, and CY-

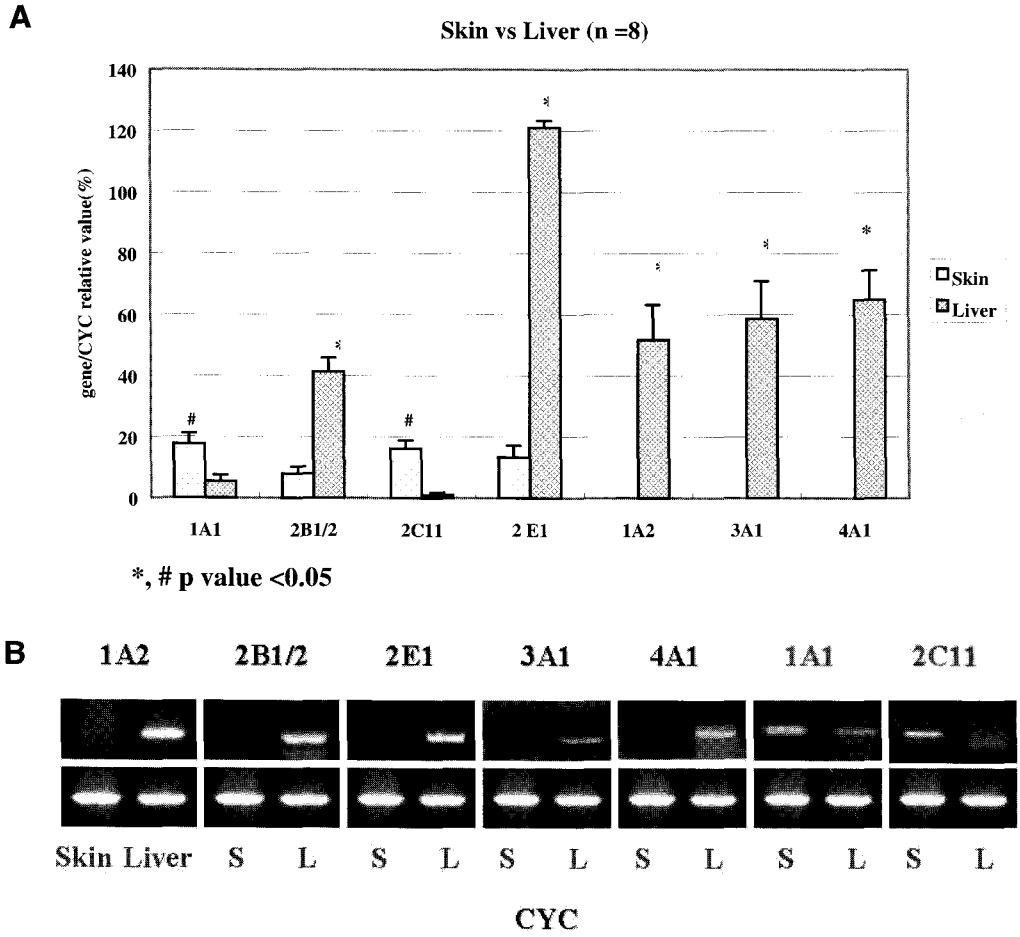

Fig. 1. Constitutive CYP isozymes mRNA expression between skin and liver. (A) RT-PCR of the skin and liver with primers specific for CYP isozymes and cyclophyllin (CYC) in 8 Sprague-Dawley rats without vehicles. The constitutive mRNA levels of CYP1A2, CYP2B1/2, CYP 2E1, CYP3A1, and CYP4A1 in the liver (*) were significantly higher than those in the skin. On the contrary, the expression of CYP1A1 and CYP2C11 were higher in skin (#). (B) Expression of CYP isozymes was clearly different between liver and skin. Expression of CYC mRNA is displayed as a loading control.

P4A1 mRNAs in the liver were 51.8±32.2, 41.2±13.1, 121.0±6.6, 58.6±35.0, and 64.9±27.5, respectively, which were significantly higher than those in the skin ($p<0.05$). The levels of cutaneous CYP 2B1/2 and CYP2E1 were 7.9±6.5 and 13.3±10.9. These levels were less than 20% of hepatic levels.

CYP1A2, CYP3A1, and CYP4A1 were not expressed in the skin.

On the contrary, constitutive expression of CYP 1A1 and CYP2C11 were higher in skin (p=0.017 and 0.012, respectively). The levels of cutaneous CYP1A1 and CYP2C11 were 17.7±9.9 and 16.1±8.0, while hepatic CYP1A1 and CYP2C11 were 5.4±6.0 and 0.9±2.6.

Cutaneous CYP isozymes mRNAs after topical white petrolatum (Fig. 2A, 2B)

Topical application of white petrolatum induced changes of mRNA expression. The same cutaneous isozymes such as CYP1A1, CYP2B1/2, CYP2C11, and CYP2E1, which expressed constitutively, were induced. Among them, the levels of CYP1A1 and CY P2C11 mRNAs were induced significantly (p=0.012 and 0.017, respectively) after applying white petrolatum (67.4±26.6 and 30.7±24.8).

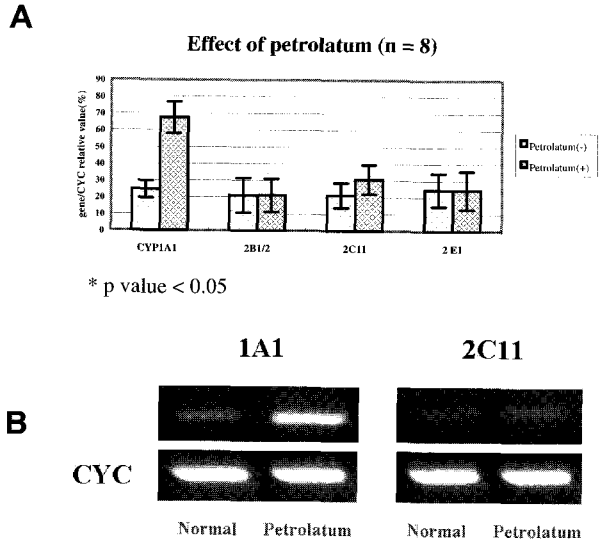

Fig. 2. CYP isozymes mRNAs change by petrolatum in rat skin. (A) Topical applicaton of petrolaum induced the same cutaneous isozymes such as CYP1A1, CYP2B1/2, CYP2C11, and CYP2E1, which expressed constitutively on the skin. Among them, the levels of CYP1A1 and CY P2C11 mRNAs were significant. (B) RT-PCR with specific primers for CYP1A1, CYP2C11 and CYC. mRNAs of CYP1A1 and CYP11 were induced with topical petrolatum.

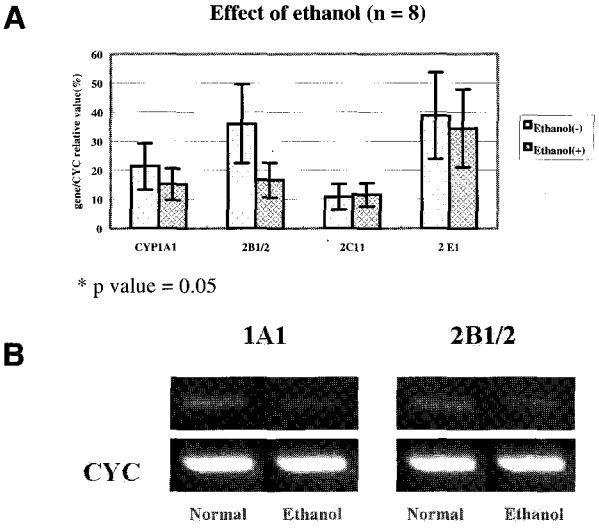

Fig. 3. CYP isozymes mRNAs change by ethanol in rat skin. (A) Topical ethanol did not make changes the levels of CYP2C11 and CYP2E1. However, the levels of CYP 1A1 and CYP2B1/2 mRNAs were decreased significantly. (B) Expression of CYP1A1 and CYP2B1/2 mRNAs was decreased with ethannol application.

Cutaneous CYP isozymes mRNAs after topical DMSO

Application of DMSO did not make any changes in isozymes mRNAs expression.

Cutaneous CYP isozymes mRNAs after topical ethanol (Fig. 3A, 3B)

Absolute ethanol also induced changes of mRNA expression in the rat skin. The induced isozymes were similar to those from a white petrolatum. The levels were not changed in CYP2C11 and CYP2E1. CYP 1A1 and CYP2B1/2 mRNAs were decreased significantly (p=0.05 and 0.012, respectively).

DISCUSSION

The level of cutaneous CYPs is approximately 5% of the hepatic levels, with requiring a sensitive method for the detection. The results may be different depending on the methods of sensitivity (Vizethum et al, 1980; Khan et al, 1989; Pham et al, 1989). An extensive study of cutaneous drug metabolizing enzyme showed that rat skin microsomes contained CYP2B1and CYP1A1 (Pham et al, 1989). Besides CYP2B1/2 and CYP1A1, CYP2C11 and CYP2E1 isozymes mRNAs were also expressed in our rats skin, which was examined by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) (Morris & Davila, 1996).

The pattern and level of mRNA expression varied in different organs (Gonzalez, 1992). Each rat skin expressed three to four kinds of isozymes such as CYP1A1, CYP2B1/2, CYP2C11, and CYP2E1. Constitutive expression of CYP isozymes mRNAs were usually higher in the liver as expected. However, CYP1A1 and CYP2C11 were higher in the skin (Fig. 3A).

CYPs are inducible enzymes. Not only systemic administration (Merk et al, 1989; Goerz et al, 1994; Tsambaos et al, 1994) but also topical application of drugs (Goerz et al, 1995) induced cutaneous and hepatic CYPs. Popular vehicles such as white petrolatum, DMSO, and ethanol also made the change of cutaneous mRNA expression.

CYP1A1 is one of the well-known isozymes, which can be induced in rat skin and liver by certain drugs. White petrolatum (petrolatum jelly) contained

polycyclic aromatic hydrocarbon (Mehrotra et al, 19 87) and CYP1A1 played a major role in the bioactivation of procarcinogens, polycyclic aromatic hydrocarbons (Vizethum et al, 1980; Ichikawa et al, 1989; Khan et al, 1992), dioxin (Kedderis et al, 1993; Christou et al, 1994) and β-naphthoflavone (Raza et al, 1992; Raza & Mukhtar, 1993; Stauber et al, 19 95). Therefore, induction of CYP1A1 could be expected with a white petrolatum in our results.

Besides CYP1A1, white petrolatum induced another isozyme, CYP2C11. The pattern of changes were different in the other vehicles: ethanol inhibited expression of CYP1A1 and CYP2B1/2. The effects of ethanol on CYP activities in human microsomes (Busby et al, 1999) were similar to our results, with displaying an inhibition of CYP1A1 and CYP2B6 at the concentration of 0.1%. DMSO did not induce or inhibit above isozyme mRNAs, although DMSO has been reported to inhibit CYP3A4, CYP2C19, and CYP2D6 in human (Busby et al, 1999).

Skin testing has been used for the identification of offending drugs in drug eruptions, and patch testing is one of them. Skin tests are methods for drug administration through a topical route. Positive reactions could be expected from skin testing, if similar allergens would be made from both systemic and topical routes of administration. These three chemical agents have been popularly used for patch testing as vehicles. However, appropriate vehicles for the testing have been so far undetermined and controversial. The effects of each vehicle on the expression of CYP isozymes mRNAs were different. Although the results were obtained from semi-quantitative RT-PCR with need for a further study, the results suggested that vehicles could influence on the cutaneous drug metabolism and an appropriate vehicle for patch testing could vary in different drugs.

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