Short Communication

Presence of Cytochrome P450 Enzymes in Human CD34$^+$ Haematopoietic Progenitor Cells

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Abstract. Human CD34$^+$ cells (haematopoietic stem and progenitor cells separated from peripheral blood) were shown to express CYP2E1 protein by Western blotting. For the first time, the specific CYP2E1 activity (chlorzoxazone 6-hydroxylation) was also detected. No CYP3A4 protein neither the CYP3A-specific nifedipine oxidase activity were detectable. The results obtained indicate differences in content of active CYP proteins in populations of stem and progenitor cells from different species as the CYP3A2 (a rat form similar to human CYP3A4) was shown to be expressed in bone marrow derived cells by RT-PCR (Avital et al. 2001).

Key words: Cytochromes P450 — CYP2E1 — CYP3A4 — Haematopoietic stem cells — Progenitor cells

One of the most conserved enzyme activities of organisms is their ability to convert relatively nonpolar substrates in the more polar ones important for biosynthesis of active compounds (e.g. the steroid hormones) or, into their derivatives which may be more easily excreted from the body, by cytochromes P450 (CYP) (Ortiz de Montellano 1995). CYP are the most abundant proteins in liver microsomal fraction. Among mammals, CYP2E1 belongs to the most structurally conserved CYP enzymes; recent investigations indicate its links to lipid and sugar metabolism (Lieber 1997) as well as its role in the brain e.g. in regulation of levels of dopamine (Nissbrandt et al. 2001). The most important human cytochrome P450 is CYP3A4, metabolizing over 50% of drugs biotransformed by CYP enzymes. The second most important family of drug metabolizing CYP enzymes in

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human is the CYP2C one with the CYP2C9 enzyme being the most interesting as it metabolizes, e.g., the anticoagulant warfarin or oral antidiabetics (Anzenbacher and Anzenbacherová 2001). This is why we focused on the detection of CYP2E1, CYP3A4 and CYP2C9 proteins in haematopoietic progenitor cells and on their function by using of the specific substrates.

Haematopoietic stem cells are potential source of hepatic cells as they were shown to differentiate into hepatocytes *in vivo* in the mouse (Lagasse et al. 2000) as well in the man (Alison et al. 2000). Also, the ability of human bone marrow primitive, multipotent adult progenitor cells to differentiate into functional hepatocyte-like cells has been documented recently (Schwartz et al. 2002). The use of haematopoietic stem and progenitor cells for autologous transplantation after application of mobilization regimen from peripheral blood in treatment of haematological malignities is well established. These findings hence open the possibility for the use of haematopoietic stem cells in hepatology.

The presence of functional CYP protein is the definite proof of the ability of the cell to act as a fully competent entity. In human hepatocytes, all the CYP proteins listed here are present and active. On the other hand, in human haematopoietic stem cells, although their ability to differentiate into functional hepatocytes has been shown (Alison et al. 2000), the presence or absence of these proteins has not been conclusively confirmed. In the work of Bernauer et al. (2000), expression of CYP2E1 in bone marrow cells from rat, rabbit and human origin was detected by Western blotting; however, the Western blot revealed greater number of bands. The activity of human CYP2E1 was not checked there due to experimental conditions. Avital et al. (2001) claimed to detect rat CYP3A2 mRNA (rat form similar to human CYP3A4) in bone marrow derived hepatocyte stem cells; in multipotent adult progenitor cells from rat and human bone marrow, the CYP2B9, CYP2B13 (rat) and CYP1B1 (human) mRNAs were detected by PCR (Schwartz et al. 2002). This study is aimed at finding and confirming the activity of CYP proteins in $CD34^+$ cells (i.e. haematopoietic stem and progenitor cells) isolated from peripheral blood.

Cells and reagents

Human $CD34^+$ cells (haematopoietic stem and progenitor cells) were obtained from fraction of mononuclear leukocytes of peripheral blood of patients ($n = 3$, males) indicated for autologous transplantation of stem cells after treatment with granulocyte colony-stimulating factor (G-CSF). The isolation of highly purified $CD34^+$ pool of cells was performed by two-step magnetic bead cell sorting using a Miltenyi MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) separation unit with final purity of more than 96 % of $CD34^+$ cells assayed by flow cytometry. The cells (taken as control samples) were kept in phosphate-buffered saline (PBS) in ice or stored at −70 °C. The procedures associated with autologous transplantations were approved by local Ethical committee. The reagents used for magnetic sorting were obtained from Miltenyi. All other chemicals were of the highest purity and were supplied by Sigma–Aldrich (Prague branch, Czech Republic).

Western blotting

For immunostaining of the CYP2E1, CYP3A4 and CYP2C9 proteins by Western blotting, the samples were thawed (if frozen), and lysed by sodium dodecyl sulfate (SDS) containing sample buffer for electrophoresis (62.5 mmol/l tris(hydroxymethyl) aminomethane HCl, pH 6.8, 10% (v/v) glycerol, 4% (v/v) mercaptoethanol, 1% (w/v) bromophenol blue with 2% (w/v) SDS). The samples were first subjected to electrophoresis on polyacrylamide gel (8% w/w) using a BioRad (Hercules, CA, USA) MiniProtean electrophoresis apparatus with Mini Trans-Blot electrophoretic transfer cell. For immunostaining, a Gentest (Woburn, MA) anti-human CYP2E1, CYP3A4 and CYP2C9 antibodies were used with an Immun-Star (BioRad) chemiluminiscence detection of respective conjugates with alkaline phosphatase. According to the producer (Gentest), the antibodies give selective reactions with the respective human CYP enzymes to achieve the highest available specificity of detection.

Activity assays

The cells were sonicated prior to incubation for 10 s using a Sonic Dismembrator (Dynatech, Farmingdale, NY, USA) adjusted to 30% energy output. Activity of the CYP2E1 enzyme was determined by monitoring the chlorzoxazone 6-hydroxylation according to Lucas et al. (1993). The assay was performed using a Shimadzu Class VP HPLC system (Shimadzu, Tokyo, Japan) with a SPD-M10Avp UV diode array detector of the same origin. The chlorzoxazone standard was obtained from Sigma-Aldrich and the 6-hydroxychlorzoxazone was purchased from Ultrafine Chemicals (Salford, UK). The assays for the CYP3A4 activity (as nifedipine oxidase) and the CYP2C9 activity (diclofenac 4'-hydroxylation) were performed according to established procedures (by Guengerich et al. 1986, Crespi et al. 1998) using the same equipment as described above.

The results of the Western blotting revealed the presence of CYP2E1 protein in samples of the lysate of the $CD34^+$ cells (Fig. 1). Human liver microsomes were used as a positive control; here, two bands appeared as the CYP2E1 antibodies although primarily highly selective for CYP2E1 protein can recognize (when more protein is applied onto the gel) also the CYP2C protein (Souek et al. 1995). The single band in the lane of the $CD34^+$ cell homogenate documents the presence of the respective CYP2E1 protein. In the lanes with the CYP3A4 antibodies the lane with the human liver microsomes shows single band of the CYP3A4 protein, however, no trace of CYP3A4 or structurally similar (CYP3A5, CYP3A7) protein can be found in the lysate of the $CD34^+$ haematopoietic cells.

To confirm the presence of the CYP2E1 enzyme, determination of a specific CYP2E1 activity, the chlorzoxazone 6-hydroxylation was performed. This activity was shown to be highly specific for the CYP2E1 enzyme (Yamazaki et al. 1995) although recently, an indication of an involvement of the CYP3A enzymes in this reaction has been published (Gorski et al. 1997). This is not the case of the cells studied in this work as it has been shown here that the cells do not possess a CYP3A

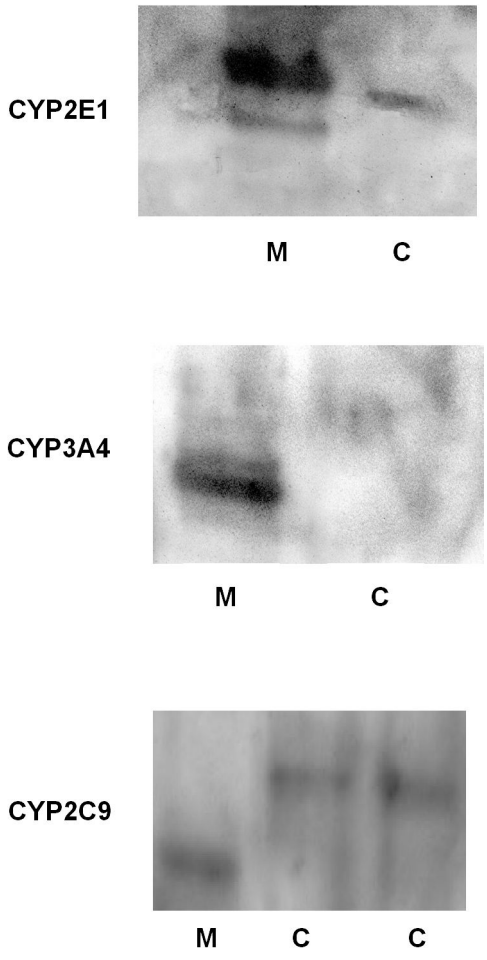

Figure 1. Western blots of CD34$^+$cell lysates with antibodies against the CYP2E1, CYP3A4 and CYP2C9 proteins. Lane M, human liver microsomes (5 μg protein); lane C, cell lysate (corresponding to 5×10^4 cells/lane).

protein (*vide infra*). An involvement of CYP1A1 in metabolism of chlorzoxazone has been implicated earlier (Carriere et al. 1993), however, the value of the Michaelis constant (Km) for this reaction is relatively high (higher values of Km indicate less specific interaction) and the selectivity for CYP2E1 has been lately confirmed (Yamazaki et al. 1995). The respective chromatogram is shown in Fig. 2. The activities ranging from 5 to 25 pmol product/min/mg protein were obtained with three different CD34$^+$ samples documenting conclusively the presence of active CYP2E1 protein in the cells.

Fig. 1 shows also the results of the search for a CYP2C9 protein by Western blotting. The specific antibody recognized the CYP2C9 protein in the sample of human liver microsomes, as well as in the lysate of the CD34$^+$ cells. However, the apparent molecular weight of the CYP2C9 protein in the cell lysate corresponds to

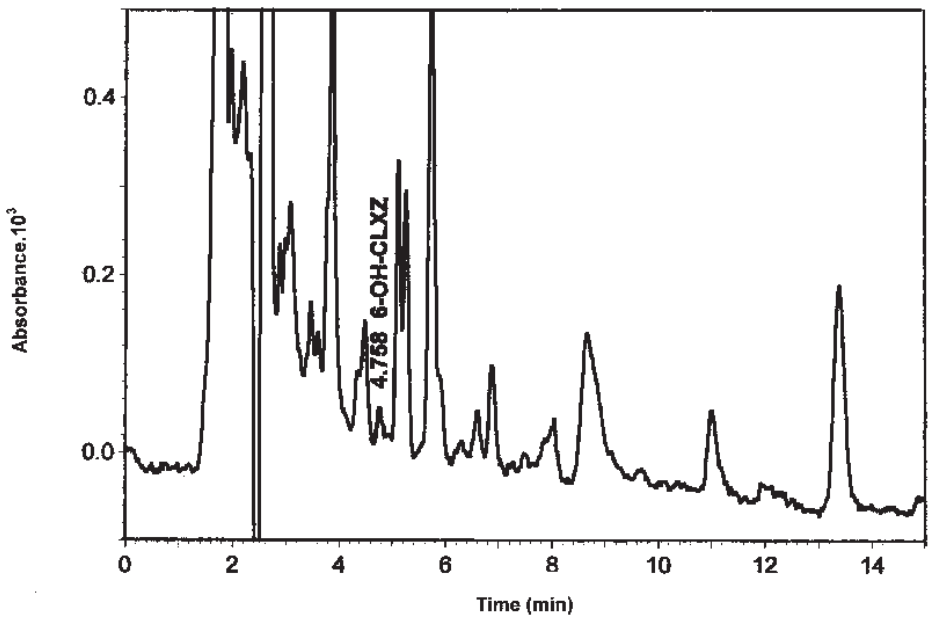

Figure 2. HPLC analysis of products of reaction mixture with chlorzoxazone to determine the CYP2E1 enzymatic activity in $CD34^+$ cell lysate. Product formed, 6-hydroxychlorzoxazone (6-OH-CLXZ). Reaction mixture (with 0.07 mmol/l chlorzoxazone, 225 μg protein) was incubated for 20 min according to Lucas et al. (1993).

a protein with higher molecular weight. Also, there was no corresponding CYP2C9 specific activity (diclofenac 4′-hydroxylation) found in the same samples. Taken together, the results may indicate that the CYP2C9 protein, if present in the cell lysate, is not in its fully competent form able to metabolize the substrates.

The results confirm for the first time the presence of an active CYP2E1 protein in human haematopoietic $CD34^+$ cells. This is in accordance with earlier indication that this protein may be found there (Bernauer et al. 2000). However, we were not able to find detectable levels of the CYP3A proteins in these cells. On the other hand, the rat bone marrow-derived stem cells seem to express a similar rat CYP3A form, CYP3A2, by PCR (Avital et al. 2001). This may indicate a possible difference between finding of an mRNA in the sample and detection of an fully competent protein there. It may well be possible that even if an mRNA (or even a protein, as it is possibly the case of CYP2C9) for a particular protein is detected, a fully competent protein is still not present and may appear lately during differentiation.

The presence of cytochromes P450 is one of the basic characteristics of the hepatic cell. Hence, it is interesting to follow the expression and maturation of these enzymes during the cell ontogenesis. The finding of at least some of them (func-

tional CYP2E1, and probably of a CYP2C9 protein) in the $CD34^+$ cell population may indicate their role in early stages of stem cell differentiation.

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