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**Note**

# High-throughput screening of anti-lipidemic agents using PXB-cells®, human primary hepatocytes from humanized mice livers: Assessment of lipoproteins by an enzyme-linked immunosolvent assay on apolipoproteins.

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We previously assessed anti-lipidemic activity by evaluating lipoprotein profiles in PXB-cells®, human primary hepatocytes from humanized mice livers, using LipoSEARCH®. This highly sensitive assay system detects low levels of extracellular lipoproteins; however, an increased throughput is needed to simultaneously assay multiple analytes. We herein investigated whether an enzyme-linked immunosolvent assay (ELISA) on apolipoproteins (APO) accurately detects reductions in extracellular lipoprotein levels in PXB-cells treated with fenofibrate as an alternative to LipoSEARCH. These results suggest the applicability of ELISA on APO to the conventional screening of anti-lipidemic activities in a large number of candidates.

**Key words:** PXB-cells®; human primary hepatocyte; lipoprotein; immunoassay; apolipoprotein; high-throughput

## Introduction

Dyslipidemia, which includes hypercholesterolemia and hypertriglyceridemia, is characterized by abnormal plasma lipid levels and increases the risk of serious disorders, such as cardiovascular disease. Therefore, the development of anti-lipidemic agents is needed. Experimental animals have been used to screen anti-metabolic syndrome activities; however, this is expensive and restrictive due to animal rights and species differences in lipoprotein profiles. We developed a novel assay system for assessing anti-lipidemic agents based on lipoprotein profiles in HepG2 human hepatoma cells using LipoSEARCH® [1, 2]. We subsequently demonstrated that lipoprotein profiles in hepatoma cell lines, including HepG2, were not an exact match to those from actual human liver tissues. Normal hepatocytes mainly secrete very-low-density lipoproteins (VLDL), similar to actual liver tissue, whereas hepatoma cell lines produce low-density lipoproteins (LDL) as the main triglyceride-rich lipoprotein [3]. We more recently reported that PXB-cells®, human primary hepatocytes from humanized mice livers, produce high levels of VLDL, and, thus, are more

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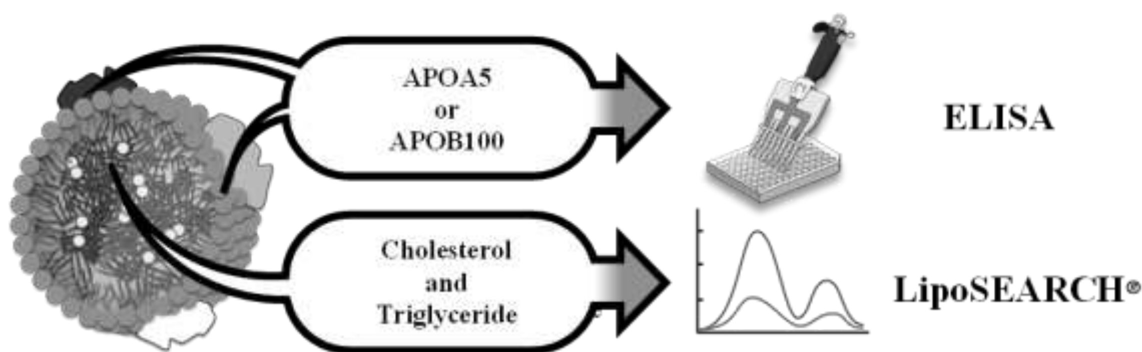


**Fig. 1 Lipoprotein assays with ELISA and LipoSEARCH.**

suitable for lipoprotein assays [4]. LipoSEARCH, the sensitivity and separation performance of which are both high, detects low levels of lipoproteins in the culture medium of cells; however, an analysis by LipoSEARCH requires more than 25 min, which is not suitable for high-throughput screening. Since lipoproteins comprise individual apolipoproteins (APO) on their surfaces depending on their classes, we herein investigated whether APO may be utilized to screen anti-lipidemic activity as an alternative to LipoSEARCH by assessing lipoproteins in PXB-cells (Fig. 1).

## Materials and Methods

*Cells and lipid assay*

PXB-cells were isolated from humanized murine livers 16 weeks after transplantation according to a previously described procedure [5], seeded at $7 \times 10^4$ cells on collagen-coated 96-well microplates (Day 0), and maintained in Dulbecco's modified Eagle medium supplemented with 10% FBS, 20 mM HEPES, 15 μg/mL L-proline, 0.25 μg/mL insulin, 50 nM dexamethasone, 44 mM $NaHCO_3$, 5 ng/mL EGF, 0.1 mM ascorbic acid 2-phosphate, 2% DMSO, and antibiotics. Cells on Day 13 in 96-well microplates were incubated in 150 μL of William's E medium supplemented with CM-4000 (Thermo Fisher Scientific, Waltham, MA, USA), respectively, with or without various concentrations of fenofibrate, an anti-lipidemic agent, for 2 days. Culture media were stored at -80°C for analyses, and subjected to an extracellular lipoprotein assay using LipoSEARCH or ELISA. Cell lysates were prepared using RIPA buffer (Nacalai Tesque, Kyoto, Japan), and protein assays were performed using Quick Start Bradford (Bio-Rad Laboratories, Hercules, CA, USA).

*ELISA*

APOA5 (a component of VLDL) and APOB100 (a component of VLDL/LDL) in the culture media of PXB-cells were measured using the APOA5 and APOB100 human ELISA kits (Immuno-Biological Laboratories, Fujioka, Japan) according to the laboratory procedure described in each manual.

*Statistical analysis*

Data are expressed as the mean ± standard deviation (SD). The significance of differences was analyzed using the Kruskal-Wallis test with the Steel test for multiple comparisons. A value of $P<0.05$ was considered to be significant.

**Table 1 Effects of fenofibrate on lipoprotein components in PXB-cells**

| | Fenofibrate (μM) | | | |
|---|---|---|---|---|
| | 0 | 50 | 100 | 200 |
| Cholesterol (μg/mg protein) | | | | |
| extracellular | 4.0± 0.6 | 2.8±0.3* | 2.5±0.3* | 1.5±1.0* |
| VLDL fraction | 3.1± 0.4 | 2.3±0.2* | 1.9±0.3* | 1.0±0.7* |
| LDL fraction | 0.6±0.2 | 0.3* | 0.3* | 0.3±0.2* |
| HDL fraction | 0.3 | 0.2±0.2 | 0.3±0.2 | 0.2±0.2 |
| Triglyceride (μg/mg protein) | | | | |
| extracellular | 34.5±5.6 | 26.7±1.2* | 20.5±4.4* | 10.3±1.2* |
| VLDL fraction | 30.8±5.3 | 23.6±1.1* | 18.0±3.9* | 8.5±1.0* |
| LDL fraction | 3.1±0.4 | 2.4±0.2 | 2.0±0.5* | 1.5±0.2* |
| HDL fraction | 0.6 | 0.7 | 0.5±0.2 | 0.4±0.3 |
| APOA5 (ng/mg protein) | 31.8±2.4 | 30.6±1.1 | 24.2±6.9* | 16.3±1.8* |
| APOB100 (μg/mg protein) | 4.4±0.3 | 4.5±0.7 | 3.4±0.6* | 2.2±0.1* |

PXB-cells at a density of $7 \times 10^4$ cells/well (150 μL) were precultured in collagen-coated 96-well microplates for 13 days after isolation and then incubated in William's E medium with CM4000 for 48 h. Cholesterol, triglyceride, and APO levels in culture media were measured (n = 4, * $P<0.05$ vs untreated cells).

## Results and discussion

LipoSEARCH is generally performed using hepatic cells cultured in 24-well microplates; however, the minimization of the assay system is important for high-throughput screening. Therefore, we examined the abilities of LipoSEARCH and its alternative method, ELISA on APO to assess the suppressive effects of fenofibrate on lipoproteins in a PXB-cell culture using 96-well microplates. The results obtained showed that both assays had the ability to evaluate the anti-lipidemic activity of fenofibrate in PXB-cells (Table 1). These results demonstrated that ELISA evaluated anti-lipidemic activity with similar sensitivity to LipoSEARCH, and may be applicable to the simultaneous screening of hundreds of samples.

Therefore, ELISA on APO has an advantage in throughput over LipoSEARCH, and is suitable for the large-scale screening of anti-lipidemic activity. However, LipoSEARCH measures not only the extracellular levels of lipoproteins, but also their particle sizes [6]. Research has recently focused on enlarging VLDL and small dense LDL, the degradation product of large VLDL, due to their relationship with myocardial infarction [7]. Therefore, LipoSEARCH may be used to screen anti-lipidemic agents for improving specific subclasses of lipoproteins, including large VLDL and small dense LDL.

## Conflict of interest

Sayaka Tomatsu and Keishi Hata have no conflicts of interest. Masaki Takahashi and Masakazu Kakuni are employees of PhoenixBio, Gen Toshima, Shiho Nakagawa, and Junichiro Takahashi are employees of Skylight Biotech., and Kazuya Miyashita and Kazumi Ogura are employed by Immuno-Biological Laboratories.

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