A Novel Cellular Model for Drug-induced Adipogenesis

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ABSTRACT

Obesity is a serious public health problem and a major risk factor for cardiovascular disease, certain types of cancer, and type 2 diabetes. Hence there is a need for relevant cellular models that can be used to test for drugs that may affect adipogenesis. Currently, most cellular models utilize cultures of fibroblastic 3T3-L1 preadipocytes that can be induced to undergo adipogenesis. However the effect of other cell types on adipocyte differentiation and function has not yet been elucidated. Since hepatocytes are the major xenobiotic metabolizing cells of the body, drug testing in the presence of hepatocytes is relevant. In this study we have used a proprietary technology, developed in our laboratory, termed Integrated Discrete Multi Organ Co-culture (IdMOC™) for the co-culture of 3T3-L1 preadipocytes and hepatocyes. Using quantitative PCR for the amplification of an early marker for adipogenic differentiation, namely AP2, we show that hepatocyte presence causes an increase in 3T3-LI adipogenesis. Using Rosiglitazone as a positive control for adipogenesis and insulin sensitization, we demonstrate further increases in 3T3-LI adipogenesis in the presence of hepatocytes. To determine the specificity of Rosiglitazne action, which occurs presumably via its metabolites, N-desmethyl rosiglitazone and ρ-hydroxy rosiglitazone, an inhibitor of the major metabolizing enzyme-CYP2C8, namely trimethoprim was used. A decrease in adipogenesis in the presence of trimethoprim indicated the role of metabolism in driving drug-induced differentiation. Such a model is physiologically relevant in testing drugs that may induce adipogenesis and as such are likely to cause weight gain and other complications.

OBJECTIVE

To develop a physiologically relevant in vitro cellular model for testing drugs that may induce adipogenesis.

This work addresses the need for a multi-cellular system that can mimic cell-cell interaction and provide drug metabolism by hepatocytes in a co-culture environment.

Integrated Discrete Multi-Organ Co-culture (IdMOC™)

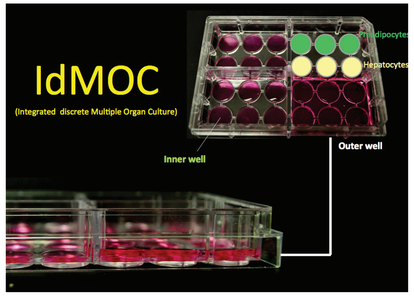
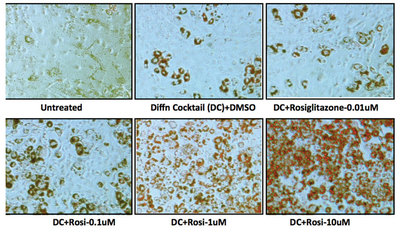


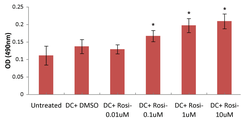
Figure 1. An IdMOC™ plate is shown seeded with 2 cell types: preadipocytes and hepatocytes in one outer well. After the cells attach the media from the inner wells is removed and the outer well is flooded with media containing the test compound under study. The flooding medium permits interconnection of multiple inner wells mimicking the integration of multiple organs via the systemic circulation.

Adipogenic Differentiation of 3T3-L1 Cells

Oil Red O staining of differentiated 3T3-LI adipocytes



Lipid quantification in 3T3-L1 adipocytes



Expression of AP2, a differentiation marker for adipogenesis, in 3T3-L1 cells

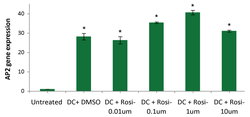


Figure 2. Adipogenic differentiation of 3T3-L1 cells. Cells (75,000/well) were treated with a differentiation cocktail (DC) containing 5μg/ml insulin, 0.25μM Dexamethasone and 500μM IBMX for a period of 3 days followed by either insulin with DMSO (DC+DMSO) or insulin with Rosiglitazone (0.01-10μM) (DC+Rosi) for the next 7 days. One set was harvested, fixed and stained with Oil Red O (Figure 2A). The lipid binding dye was then extracted and quantitated at 490nm (Figure 2B). Another set of cells were harvested, lysed and the extracted RNA was reverse transcribed and quantified for the expression of mouse AP2 (adipogenic marker) using real-time PCR. Results are normalized to the housekeeping gene-M36B4 and shown as fold change over the untreated control, using the ΔΔCt method for quantiation. Oil Red O staining was performed in triplicate (±sem) and statistical significance (P<0.05) was computed by ANOVA, post-hoc Tukey's test. Gene expression was analyzed in 2 independent experiments and is shown ±sd. Results shown are mean±sem for Oil Red O staining (n=3) and mean±sd for relative gene expression (n=2). Asterisks indicate statistical significance (P<0.05) by ANOVA, post-hoc Dunnett's test on comparing to control, untreated samples.

Optimization of the Preadipocyte-Hepatocyte IdMOC™ Model: Media and Treatment Time

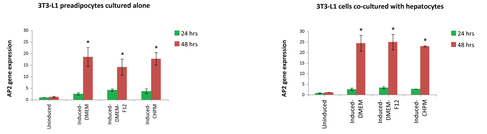
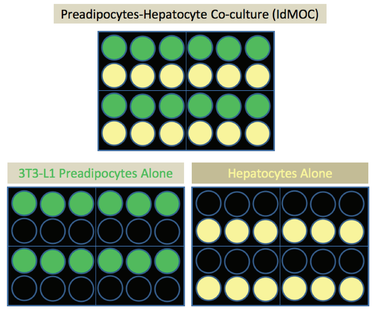


Figure 3: Optimization of the Preadipocyte-Hepatocyte IdMOC™ Model: Media and Treatment Time. 3T3-L1 cells (75,000 cells/well) were plated in DMEM in the presence and absence of hepatocytes (260,000 cells/well) which were plated in CHPM™ (Cryopreserved Hepatocyte Plating Medium, AP Sciences). After adherence the cells were treated with MatrigelTM (0.25mg/ml,BD Biosciences) which was allowed to solidy for 30 minutes. Cells were then treated with 5μg/ml insulin, 0.25μM Dexamethasone, 500μM IBMX and 10μM Rosiglitazone in either DMEM, DMEM-F12 (1:1) or CHPM™ for 24 or 48 hours. Cells were lyzed at the 2 timepoints, RNA was extracted, reverse transcribed and quantified for the expression of mouse AP2 (adipogenic marker) using real-time PCR. Results were normalized to the housekeeping gene-M36B4 and are shown as fold change over untreated/uninduced controls which were maintained in DMEM. Results shown are mean±sd of 2 independent experiments. Data shown is mean±sd (n=2) and significantly different (*) by ANOVA, Newman-Keul's Multiple Comparison Test (P<0.05) between untreated and treated samples as well as between 24 vs. 48 hr timepoints.

Experimental Design for Screening Compounds in the IdMOC™ Model



Effect of Rosiglitazone on Adipogenesis in Preadipocyte-Hepatocyte Co-cultures

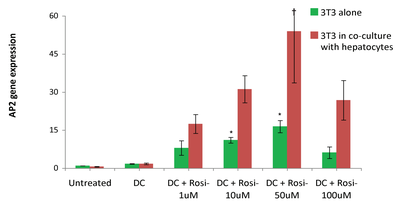


Figure 4. Hepatocytes enhance the effect of Rosiglitazone on adipogenic differentiation. 3T3-L1 cells (75,000 cells/well) were plated in the presence and absence hepatocytes (250,000 cells/well) and MatrigelTM was added at a final concentration of 0.25mg/ml. Cells were further treated with a differentiation cocktail (DC) containing 5μg/ml insulin, 0.25μM Dexamethasone and 500μM IBMX either in the presence of DMSO (vehicle control) or Rosiglitazone (1-100μM). Cells were lysed 24 hrs later and RNA was extracted, reverse transcribed and quantified for the expression of mouse AP2 (adipogenic marker) using real-time PCR. Results were normalized to housekeeping gene-M36B4 and are shown as fold change over untreated controls, as determined by the ΔΔCt method. Results shown are mean±sem of 3 independent experiments and significantly different by ANOVA, Newman-Keul's Multiple Comparison Test (P<0.05) between untreated and treated samples (*) and co-culture vs. 3T3-L1 cultured alone (†).

Effect of Rosiglitazone on the Survival of Preadipocyte-Hepatocyte Co-cultures

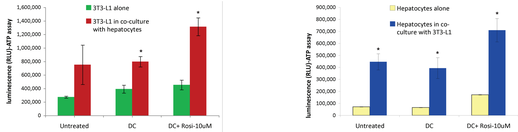


Figure 5 Reciprocal interactions between hepatocytes and preadipocytes yield increases in cellular ATP in response to Rosiglitazone. 3T3-L1 cells (75,000 cells/well) were plated in the presence and absence hepatocytes (250,000 cells/well) and MatrigelTM was added at a final concentration of 0.25mg/ml. Cells were either untreated or treated with a differentiation cocktail (DC) containing 5μg/ml insulin, 0.25μM Dexamethasone and 500μM IBMX for a period of 3 days. This medium was replaced with 5μg/ml insulin in the presence of DMSO (DC+DMSO) or 10μM Rosiglitazone (DC+ Rosi-10μM). Cells were lyzed 2 days later and cellular ATP content was quantified as relative luciferase units (RLU). Data shown is mean±sem (n=2) and significantly different (*) by ANOVA, Newman-Keul's Multiple Comparison Test (P<0.05) between co-culture and single cell- type culture samples.

Attenuation of Adipogenic Differentiation in the Presence of Trimethoprim, a CYP2C8 Inhibitor

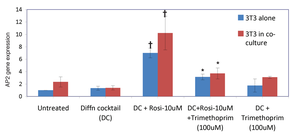


Figure 6: The effect of Rosiglitazone on adipogenic differentiation is attenuated in the presence of trimethoprim, a CYP2C8 inhibitor. 3T3-L1 cells (75,000 cells/well) were plated in the presence and absence hepatocytes (250,000 cells/well) and MatrigelTM was added at a final concentration of 0.25mg/ml. Cells were further treated with a differentiation cocktail (DC) containing 5μg/ml insulin, 0.25μM Dexamethasone and 500μM IBMX either in the presence of DMSO (DC+DMSO), 10μM Rosiglitazone (DC+ Rosi), 10μM Rosiglitazone plus 100uM Trimethoprim (CYP2C8 inhibitor) or simply DC+ Trimethoprim. Cells were lysed 48 hrs later and RNA was extracted, reverse transcribed and quantified for the expression of mouse AP2 (adipogenic marker) using real-time PCR. Results were normalized to housekeeping gene-M36B4 and are shown as fold change over untreated controls, as determined by the ΔΔCt method. Results shown are mean±sem of 3 independent experiments and significantly different by ANOVA, Newman-Keul's Multiple Comparison Test (P<0.05) between induced (DC+Rosi-10μM) and inhibited (DC+Rosi-10μM+ Trimethoprim) samples (*) as well as induced vs. untreated samples (†).

CONCLUSIONS

1. Rosiglitazone induces adipogenic differentiation of 3T3-L1 preadipocytes in the IdMOC™ system.

2. The Preadipocyte-Hepatocyte IdMOC™ model was optimized for use with a Universal media-CHPM™ over a period of 48 hrs, using quantitative expression of AP2 mRNA as the endpoint.

3. The effect of Rosiglitazone on adipogenic differentiation is enhanced in the presence of hepatocytes

4. Reciprocal interactions between hepatocytes and preadipocytes yield increases in cellular ATP in
response to Rosiglitazone.

5. The effect of Rosiglitazone on adipogenic differentiation is attenuated in the presence of trimethoprim, a CYP2C8 inhibitor. This indicates that drugs such as Rosiglitazone can induce high levels of adipogenesis on metabolism

SUMMARY

The Preadipocyte-Hepatocyte IdMOC™ model

- is a physiologically relevant model for testing drugs that can induce adipogenesis
- can be used to detect the effects of drug metabolism on adipogenesis