ABSTRACT

In-vitro and in-vivo P450 activities in hepatocytes will diminish over time in cultured hepatocytes as documented in studies using rat cultured hepatocytes and human liver slices measuring P450 activities. Other reports have included UGT and SULT activities. In this report, we have compared mRNA for human cultured hepatocytes with enzyme activity along with UGT glucuronosyltransferase (UGT) and sulforhodamine (SR) is cultured hepatocytes. Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. Enzymatic activities were determined using specific substrates including 7-hydroxycoumarin, 7-hydroxy-4-methylquinolin-3-one, 4-O-demethyltetrahydrozid, 7-HCG glucuronide, 4-OH tolbutamide, mephenytoin 4′-OH, tolbutamide 7-HC sulfate, 6β-hydroxytestosterone and 4-OH-testosterone. Data were analyzed using least-squares linear regression. Results were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

RESULTS AND DISCUSSION

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.

Enzymatic Activity Characterization:

Hepatocytes were isolated from human cadaver livers by a sequential collagenase perfusion method and cultivated in a humidified incubator at 37°C in GRO™ CP medium. The hepatocyte suspension was counted for viability and returned to 37°C, 5% CO2, humidified incubator for 30 minutes. Reactions were terminated using equal volume of methanol. Each data point was performed in triplicate. Samples were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols. Data were analyzed using least-squares linear regression. Graphs were stored at -80°C until analyzed using HPLC, LC/MS/MS or UPLC/MS/MS protocols.