ABSTRACT

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Various *in vitro* liver models play an irreplaceable role in preclinical drug evaluation. One of them is multicellular hepatic spheroids. These are 3D cell aggregates that are formed by the process of cellular aggregation. They are used in drug development and represent a promising tool for identifying new targets and drug screening for various liver diseases. The aim of this thesis was to introduce and optimize the preparation of liver spheroids by the „*forced-floating*“ method. The liver of male and female Wistar rats was used for hepatocyte isolation and spheroid preparation. Due to the low viability of isolated hepatocytes, we tried to optimize the isolation procedure. Nevertheless, we managed to obtain only a small number of hepatocytes with sufficient viability for subsequent formation of spheroids. The spheroids were incubated with various lipogenic reagents for 7 and 14 days and their viability was monitored by measuring adenosine triphosphate (ATP) production. Incubation of spheroids with lipogenic substrates for 7 days did not result in significant changes in ATP levels, while incubation of spheroids with the amiodarone alone and a combination of lipogenic substrates with insulin for 14 days resulted in a significant decrease in their viability. Albumin was used as a marker of the synthetic activity of spheroids. Using the dot blot method, we found out that the spheroids were able to synthesize albumin and secrete it into the culture medium. We also verified the ability of spheroids to express selected genes encoding biotransformation enzymes (cytochrome P450 1/2, 3A, NAD(P)H:quinone oxidoreductase 1). The results show that although their expression fluctuated, there was no significant change in mRNA expression during incubation.