Amiodarone-Induced Phospholipidosis And Steatosis In Human Hepatoma HepaRG Cells

Sébastien Anthérieu[1], Christophe Chesné[2], André Guillouzo[1]

[1]INSERM U620-MDC, Université de Rennes1, Rennes, France
[2]Biopredic International, Rennes, France

sebastien.antherieu@univ-rennes1.fr

Drug-induced liver injury is the most frequent cause cited for the withdrawal from the market of an approved drug and is also a major cause of attrition in drug development, indicating that preclinical evaluation of new drugs is of critical importance. Since most drug-induced adverse reactions usually occur after repeated treatments in few patients and are unpredictable there is a need for models that better predict and explain chronic hepatotoxicity. Unfortunately primary human hepatocytes exhibit large interdonor variability and have a too short life *in vitro* while most liver cell lines express only low levels of liver-specific functions. However, we have recently reported that a new human hepatoma cell line, named HepaRG and composed of both hepatocyte-like and biliary-like cells (50/50), has retained the major P450 activities, as well as various liver-specific functions, at levels comparable with those found in primary human hepatocytes (Aninat et al., 2006). Moreover, HepaRG cells retain relatively stable levels of cytochromes P450 expression and activities for several weeks at confluence (Jossé et al., 2008). In this study we used the well-differentiated HepaRG cell line to analyse acute (24h, one addition) and chronic (14 days, addition every 2-3 days) effects of amiodarone, a drug known to induce phospholipidosis and steatosis in the liver of few treated patients.

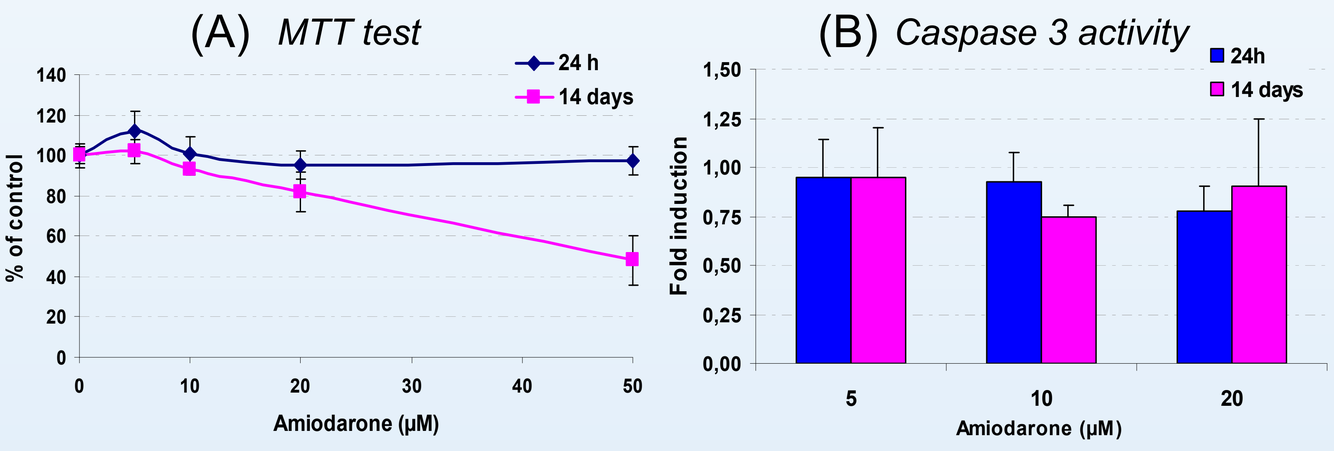

Figure 1: Comparative *in vitro* cytotoxicity of acute and chronic exposures to amiodarone in HepaRG cells. HepaRG cells were incubated for 24h (one addition) or 14 days (repeated treatment every 2-3 days) with different concentrations (0-50µM) of amiodarone. Cytotoxicity was measured by the MTT test **(A)** and apoptosis assay was performed by measuring the caspase 3 activity **(B).**

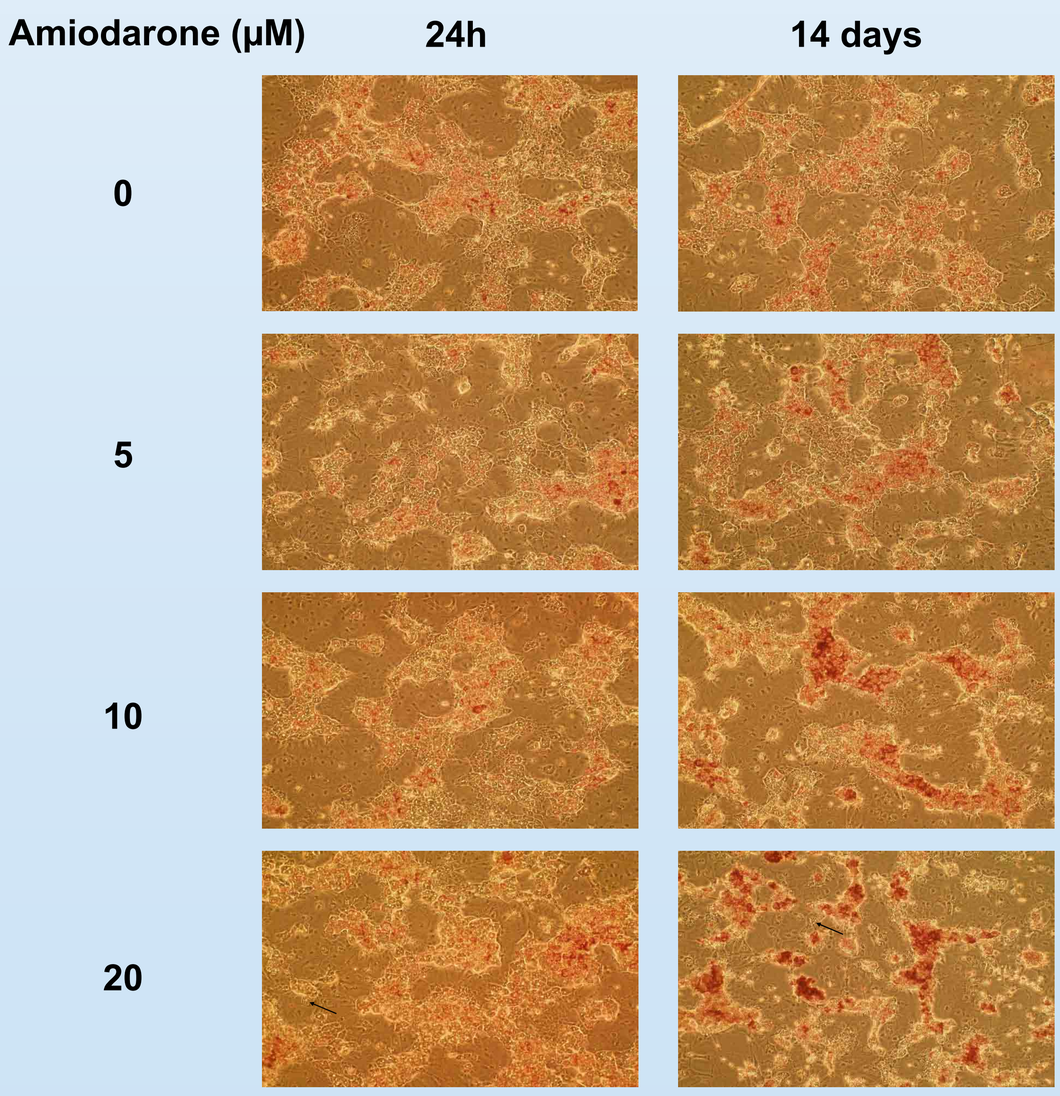

Figure 2: Light microscopic analysis of HepaRG cells treated with various concentrations of amiodarone after Oil-Red O staining. HepaRG cells were exposed for 24h or 14 days with different concentrations (0-20µM) of amiodarone. Then lipid accumulation was determined by Oil-Red O staining, which allows the detection of triglycerides and cholesterol esters. HepaRG cells were observed and photographed with phase-contrast microscope. **Labelled lipid droplets were observed in hepatocytes and not in biliary cells, only after reiterated exposure to amiodarone. Moreover amiodarone induced formation of intracytoplasmic vesicles (not stained with Oil-Red O) in the two cell types after 24 h and 14 days of treatment at 20 µM (arrows).**

References: Aninat C, Piton A, Glaise D, Le Charpentier T, Langouët S, Morel F, Guguen-Guillouzo C, Guillouzo A. Drug Metab Dispos.2006; 34:75-83.
Jossé R, Aninat C, Glaise D, Dumont J, Fessard V, Morel F, Poul JM, Guguen-Guillouzo C, Guillouzo A. Drug Metab Dispos. 2008; 36:1111-8.

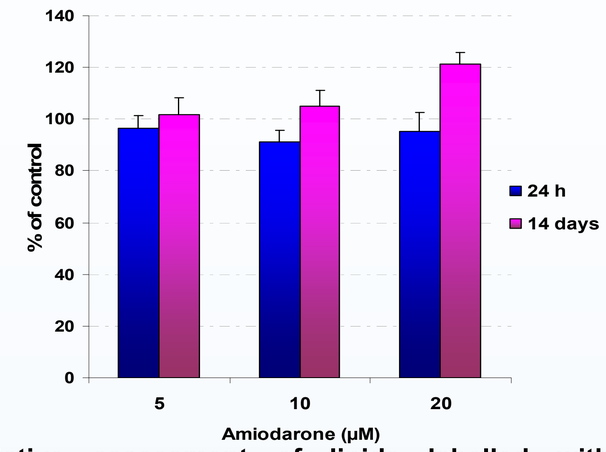

Figure 3: Quantitative assessment of lipids labelled with Oil-Red O by spectrophotometry after treatments with different concentrations of amiodarone. HepaRG cells were exposed for 24h or 14 days with different concentrations (0-20µM) of amiodarone. After incubation with Oil-Red O, DMSO was added to dissolve bound Oil-Red O and lipid accumulation was measured at 520 nm. **Chronic exposure to amiodarone induced a dose-dependent increase of lipids in HepaRG cells.**

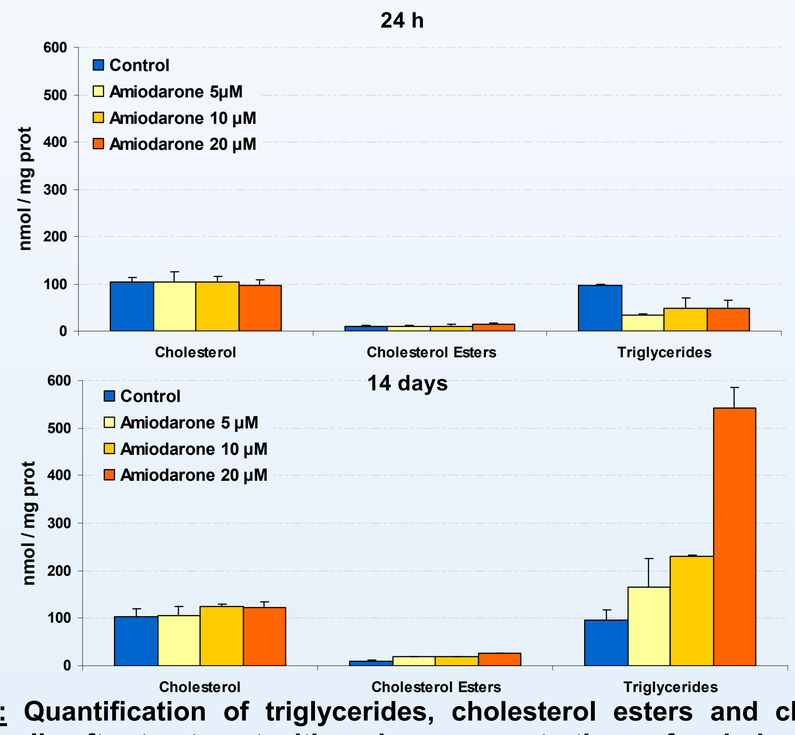

Figure 4: Quantification of triglycerides, cholesterol esters and cholesterol in HepaRG cells after treatment with various concentrations of amiodarone. HepaRG cells were exposed for 24h or 14 days to different concentrations (0-20µM) of amiodarone. Then lipids were extracted (chloroform/methanol/water in the presence of internal standards) and neutral lipids (triglycerides, cholesterol and cholesterol esters) were analysed by Gas Liquid Chromatography. **Chronic exposure to amiodarone induced a dose-dependent increase of triglycerides and more weakly cholesterol esters in HepaRG cells.**

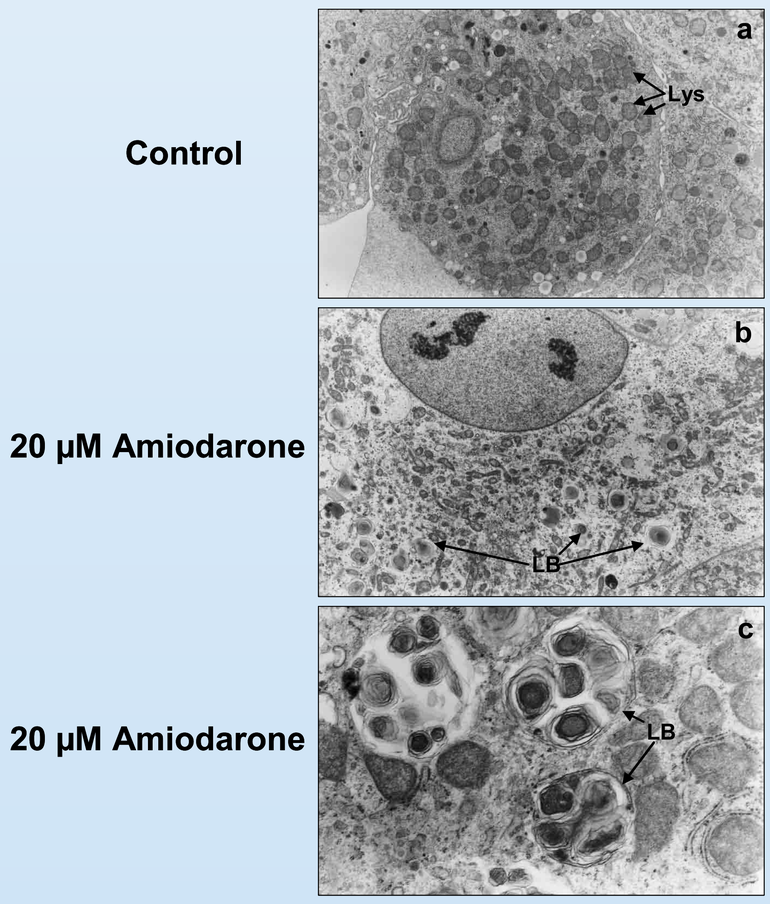

Figure 5: Electron microscopic analysis of control and amiodarone-treated HepaRG cells. HepaRG cells were incubated with solvent (control) or 20µM amiodarone for 24h, and then analysed under electron microscopy (a-b: x2700 ; c: x10000). Lys: lysosomes, LB: lamellar bodies. **Amiodarone induced the formation of lamellar bodies, the allmark of phospholipidosis.**

CONCLUSION:

Taken altogether, these results show that amiodarone induces phospholipidosis after short-term treatment (24h) and both phospholipidosis and steatosis after long-term exposure (14 days) in HepaRG cells. These findings are in agreement with observations made in patients. They support the conclusion that HepaRG cells represent a suitable *in vitro* model for studying drug-induced liver injury in human.

This study was supported by the EEC contract LIINTOP-STREP-037499