The *mini* BioArtificiaLiver: a cellular biosensor in the drug development process

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Aims

Every year a great amount of financial resources is spent by pharmaceutical companies on unsuccessful Clinical Trials because of poor activity/toxicity ratio, pharmacokinetics and pharmacodinamics accidents including poor Absorption-Distribution-Metabolism-Excretion/Toxicology profiles.
Considering the key role accomplished by the liver in toxicology and metabolism of drugs there is a great need for screening tools using human cells. Furthermore the set up of bio-systems using human cells and able to test candidate drugs could be helpful in reducing the use of animals in the experimental testing as well as lowering costs concerning the drug development process.

Methods

In the Center of Biotechnologies of Cardarelli Hospital, in Naples, in collaboration with the Academic Medical Center of Amsterdam University, we assembled a *mini Bioartificial Liver (mini BAL)* able to host approximately 300,000 millions of viable human hepatocytes. The mini BAL is a three-dimensional system which resembles the best conditions for hepatocyte culturing and, differently from other monolayer approaches, shows a system-integrated oxygenation addressed to metabolism and respiratory functions optimization. Human hepatocytes are obtained by livers discarded from transplantation as well as surgical liver resections. Considering the low availability of human liver cells and in view of our experience, we used porcine hepatocytes in order to standardize the overall process.
Previously we were able to successfully investigate in the mini BALs the effects of different doses of amphetamine, a well-known hepatotoxic drug, for 7 days.
In this experiment we assessed the function of n° 3 minibal charged with 300,000 million viable human hepatocytes (viability≥ 80%) evaluating the specific cell functions through urea production, ammonia clearance, albumin synthesis.
We observed a sharp, dose-dependent, decrease in hepatocytes functionality (urea production) with a restoration of the functionality in the following days.

Results

In the Center of Biotechnologies (Cardarelli Hospital, Naples) we have assembled, in cooperation with Amsterdam University, a *mini Bioartificial Liver (mini BAL)* able to host approximately 300,000 millions of viable human hepatocytes.
In the present study we propose to evaluate the hepatotoxicity of candidate drugs in the miniBAL environment closely resembling an *in vivo* system by utilizing a primary cell culture system in a three dimension oxygenated structure.
The miniBAL is a three-dimensional system which designs the better conditions for hepatocyte culturing and, differently from other monolayer approaches, shows a system-integrated oxygenation addressed to metabolism and respiratory functions optimization.

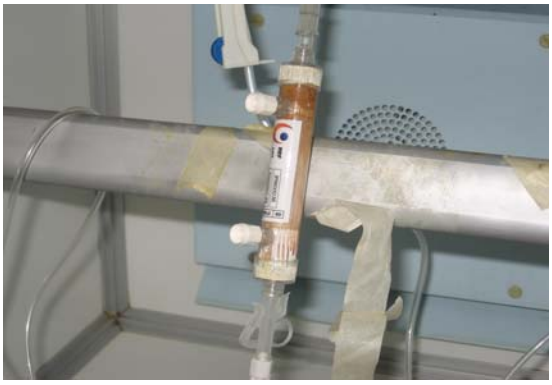

Figure 1: the miniBAL

Human hepatocytes are obtained by surgical resections (under informed consent signed by patients) or livers discarded from organ transplantation. Considering the low availability of human liver cells and in view of our experience, we firstly used porcine hepatocytes in order to standardize the overall procedure.
Our proposal is to use the miniBAL as a system able to detect CYP450-dependent biotransformation reactions of drugs and metabolites as well as for the evaluation of liver specific toxicity when exposed to different molecules. *In other words, this device can be characterized as a human cellular biosensor to evaluate the potential toxicological effects of known and unknown compounds*.
The development of this miniBAL charged with human hepatocytes, as well as the advantages in the standardization procedures of this system, could represent a very important tool to test drugs for potential use in man and to further evaluate, in a simultaneous sequence, more molecules to be assessed for potential hepato-toxic or hepato-trophic effect [(10)].
In particular we are going to investigate how much the variation in conventional biomarkers of hepatic functions (urea synthesis, ammonia clearance, cell viability, etc) is altered by hepatotoxicity-inducing compounds in the miniBAL as an in vitro cell culture system.
We have already successfully investigated the effects of administration of a well-known hepatotoxic drug, amphetamine, in the miniBAL circuit over a 5 days observation. In this experiment we assessed the function of n° 3 minibal charged with 300,000 million viable human hepatocytes (viability≥ 80%) evaluating the specific cell functions through urea production, ammonia clearance, albumin synthesis.

We observed a sharp, dose-dependent, decrease in hepatocytes functionality (urea production) with a restoration of the functionality in the following days.

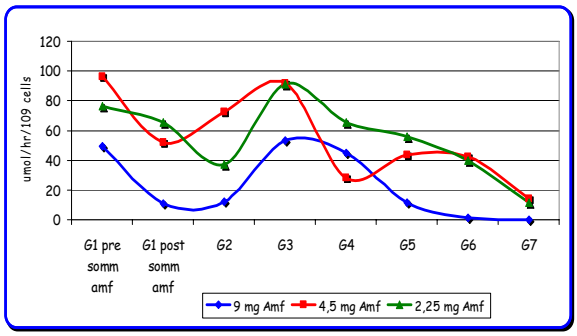

Figure 2: results about urea production

In *vitro* models of liver using isolated primary hepatocytes have been used as screening systems for metabolism evaluation, hepatocyte proliferation studies, and as bioartificial liver support .
The development of tissue and in vitro models based on human cells provides a potential bridge to the gap between animal models and human studies screening the efficacy and safety of new drugs before clinical trials**.**
We propose the use of the mini BAL system as a method between *in vitro* testing and clinical studies of promising compounds for human healthcare. Our purpose is to use the mini BAL as a system able to detect hepatocellular biotransformation reactions CYP450-dependent and to evaluate the hepatotoxicity of molecules developed for clinical application.
Moreover our report has a paramount significance related to the ethical aspects of preclinical studies. The mini BAL may allow a sensible reduction of the number of animals used in drug testing and a reliable tool for toxicological and pharmacological studies.
We are currently investigating the possibility of performing multiple toxicological profiles with hepatocytes coming from the same source targetet to reproducible and translational data , in order to use a system between *in vitro* testing and clinical studies
Additionally this system will provide long-lasting, data in a early stage ,improving the drug development process as well as the financial and human resources.
The mini bioartificial liver may allow a sensible reduction of use of animals in and can be considered as a "biological hybrid", and a powerful, and reliable tool for toxicological and pharmacological studies

Concluding:
- we have previously investigated the large size BAL as a hepatic system used in a Clinical Trial to metabolically support liver failure bridging 14 acute patients to orthotopic liver transplantation [1, 2, 3, 8,9,11,12,13,14,15].
- now we have set up the miniBAL as a substitute replacing the use of animals and representing a reproducible and reliable tool for toxicological and pharmacological studies of classic molecules and biotechnological drugs.

Conclusions

We propose the use of the mini BAL system as an ”hybrid” in the biological scale, between *in vitro* testing and clinical studies of promising compounds for human healthcare. Our proposal is to use our mini BAL as a system able to detect hepatocellular biotransformation reactions CYP450-dependent.

Finally our report has a paramount significance related to the ethical aspects of preclinical studies. The mini BAL may allow a sensible reduction of the number of animals used in drug testing and a reliable tool for toxicological and pharmacological studies.

Drug-induced liver toxicity is worldwide the leading cause of acute liver failure and post-market drug withdrawals. Every year a great amount of financial resources is spent by pharmaceutical companies on unsuccessful Clinical Trials because of poor activity/toxicity ratio, pharmacokinetics and pharmacodinamics accidents (including poor *Absorption-Distribution-Metabolism-Excretion*-ADME/toxicology profiles) as well as serious adverse effects occurring in the post-marketing *surveillance* phase.
In addition, many candidate compounds have been compelled to cease further development as possible new drugs because they lack of an exhaustive safety evaluation in the early stage of drug discovery.
The liver plays a crucial role in drug metabolism and toxicology, accomplishing many physiological functions (such as synthesis, storage, metabolism, secretion of biogenic components in the living body) as well as in cellular homeostasis. Since these functions are dependent on human parenchymal liver cells, primary cultures of these cells are suitable to be used for toxicological evaluation, such as related to hepatocytes necrosis, etc.
Many human diseases and drug toxicities are very complicated events that can be represented only in animal models, but unfortunately these models lack in human physiology. At this regard preclinical animal studies are often inadequate to evaluate toxicity because of species-specific variation between animal and human hepatocellular functions, necessitating supplementation of animal data with assays to assess human responses.
The evolution from monolayer to collagen sandwich cultures up to three-dimensional systems, able to maintain higher differentiation over a longer term, has led to the development of more suitable and reliable systems for toxicity assessment. The introduction of a compound-specific in vitro cell culture system has numerous advantages, but of most importance, the generation of sufficient results for a toxicology screening and safety evaluation at a low cost, high speed and less animal use can be expected.
More recently the need for screening tools for microscale toxicology assays is becoming greater and more pressing [4,5,6,7]. There is a real need for more predictive systems able to test candidate drugs; moreover these systems could be aimed to lower the costs of drug development process as well as to play a significant role in the 3Rs – the reduction, refinement and replacement of the use of animals in preclinical drug development research.
Pharmaceutical companies have started a toxicity evaluation of candidate drugs at a very early stage in the discovery process in order to reduce the chances of late-stage failure. . Such early-stage toxicity informations require the development of accurate, practical and reproducible in vitro assays reliable for human toxicity prediction .

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