

Measurement of the extracellular release of adenosine triphosphate in cultured primary rat hepatocytes

Commonly used acronym: ATP measurement

Created on: 12-03-2019 - Last modified on: 28-02-2022

SCOPE OF THE METHOD

The Method relates to	Animal health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
This method makes use of	Animal derived cells / tissues / organs
Species from which cells/tissues/organs are derived	rat
Type of cells/tissues/organs	primary hepatocytes

DESCRIPTION

Method keywords

ATP
cytotoxicity
bioluminescent determination
extracellular ATP

Scientific area keywords

Hepatotoxicity
liver
cholestasis
Steatosis

Method description

ATP transports chemical energy within cells by serving as a substrate for kinases and as such fulfills a vital function in numerous cellular processes such as cell injury and subsequent cell death. ATP is therefore a crucial player in these events that are results of intracellular stress. Hepatotoxic chemical compounds can cause intracellular stress. The general cytotoxicity of compounds can be estimated through the bioluminescent assessment of extracellular release of ATP. As such, this procedure relies on two reactions in which firefly luciferase catalyzes the oxidation of luciferin to oxyluciferin and whereby ATP is consumed and light becomes emitted. The latter can be measured and is proportional to the amount of ATP present outside cells.

Lab equipment

Multiplate reader (Victor, 1420 Multilabel counter, PerkinElmer, Belgium)

Method status

History of use

PROS, CONS & FUTURE POTENTIAL

Advantages

Easy to apply method to quantitatively characterize extracellular ATP release and hence cell injury in primary hepatocyte cultures.

Challenges

Increased extracellular levels of ATP do not specifically indicate cell death by either apoptosis or necrosis. Ideally this method should be combined with established tests, such as the monitoring of cell proliferation potential and mitochondrial function, which can be done by measurement of the incorporation of 5-bromo-2'-deoxyuridine (BrdU) during DNA synthesis and by addressing an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, respectively.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

- Eguchi Y., Shimizu S. and Tsujimoto Y. (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Research* 57:1835-1840
- Elaut G., Henkens T., Papeleu P., Snykers S., Vinken M., Vanhaecke T. and Rogiers V. (2006) Molecular mechanisms underlying the dedifferentiation process of isolated hepatocytes and their cultures. *Current Drug Metabolism* 7: 629-660
- Golstein P. and Kroemer G. (2007) Cell death by necrosis: towards a molecular definition. *Trends in Biochemical Sciences* 32: 37-43

Gómez-Lechón M.J., Lahoz A., Gombau L., Castell J.V. and Donato M.T. (2010) In vitro evaluation of potential hepatotoxicity induced by drugs. *Current Pharmaceutical Design* 16: 1963-1977

Jaeschke H., Gores G.J., Cederbaum A.I., Hinson J.A. Pessayre D. and Lemasters J.J. (2002) Mechanisms of hepatotoxicity. *Toxicological Sciences* 65: 166-176. McKim J.M. (2010) Building a tiered approach to in vitro predictive toxicity screening: a focus on assays with in vivo relevance. *Combinatorial Chemistry and High Throughput Screening* 13: 188-206

Papeleu P., Vanhaecke T., Henkens T., Elaut G., Vinken M., Snykers S. and Rogiers V. (2006) Isolation of rat hepatocytes. *Methods in Molecular Biology* 320: 229-237

Seglen P.O. (1976) Preparation of isolated rat liver cells. *Methods in Cell Biology* 13: 29-83

Tsujimoto Y. (1997) Apoptosis and necrosis: intracellular ATP level as a determinant for cell death modes. *Cell Death and Differentiation* 4: 429-434

Van Calsteren V. (2010) Analyse van de herhaalde toedieningstoxiciteitstesten met cosmetische ingrediënten bestudeerd op Europees niveau. Dissertation Master in Pharmaceutical Sciences - Vrije Universiteit Brussel, Rogiers V. (promotor) and Pauwels M. (co-promotor)

Vinken M., Decrock E., De Vuyst E., Leybaert L., Vanhaecke T. and Rogiers V. (2009) Biochemical characterisation of an in vitro model of hepatocellular apoptotic cell death. *Alternatives to Laboratory Animals* 37: 209-218

Associated documents

PARTNERS AND COLLABORATIONS

Organisation

Name of the organisation Vrije Universiteit Brussel (VUB)

Department Pharmaceutical and Pharmacological Sciences

Specific Research Group or Service In Vitro Toxicology and Dermato-Cosmetology

Country Belgium

Geographical Area

Brussels

Region

Coordinated by



Financed by

