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Attention NTP Interagency Center for the Evaluation of Alternative Toxicological Methods Federal Register Notice: (81FR42718-42719)

Name: Sogorb, Miguel A

Affiliation Type: Academia

Affiliation: Bioengineering Institute, Miguel Hernandez University

Comments Submitted on Behalf of a Sponsoring Organization?: No

Organization Name: Miguel Hernandez University

Comments: We developed a methodology for predicting embryotoxicity of chemicals on the basis of Embryonic Stem cell Test (EST). We enhanced the performance of the classical EST as follows: i) introducing molecular end-points for improving the monitoring of the alterations in the differentiation of embryonic stem cells; ii) reducing the total length of the test to 5 days (half of the standard procedure); and, iii) simplifying the procedure by the use of only monolayer cell cultures avoiding the laborious handling of hanging-drops embryonic bodies and the difficult assessment of the beating of the differentiated cardiomyocytes.

The molecular end-point introduced in the procedure was the expression of sixteen different biomarkers genes, four of ectoderm (Nrcam, Nes, Shh and Pnpla6), two of endoderm (Flk1 and Afp), four of mesoderm (Mesp1, Vegfa, Myo1e and Hdac7) and six general cellular processes (Cdk1, Myc, Jun, Mixl, Cer and Wnt3).

The model chemicals for testing the performance of our methodology were 5-fluorouracil and retinoic acid as strong embryotoxicants (those able to induce embryotoxicity in vivo without maternal toxicity); 5,5-diphenylhydantoin, LiCl and valproic acid was weak embryotoxicants (those able to induce embryotoxicity in vivo only concurrently with low or mild maternal toxicity) and penicillin G and saccharin as non-embryotoxicants (those that only induce embryotoxicity concurrently with severe maternal toxicity or lethality).

The cellular models were the same used for standard EST. Thus, we used 3T3 mouse fibroblasts as model of non-differentiating cells and determined IC503T3 as the concentration of the tested compound that causes a decrease of 50% of viability in 3T3 cells after 5 days of exposure. We also used D3 mouse embryonic stem cells as model of differentiating cells and determined two different end-points: i) IC50D3 as the concentration of the tested compound that causes a decrease of 50% of viability in D3 cells after 5 days of exposure; and, ii) either ECD200, as the effective concentration of the tested chemical that causes an increase of 200% in the expression of the biomarker gene in spontaneously differentiated cells after 5 days of exposure; or ECD50, as the effective concentration of the tested chemical that causes a decrease of 50% in the expression of the biomarker gene in spontaneously differentiated cells after 5 days of exposure.

At the end, we applied the prediction models developed for classical EST for discriminating among strong, weak and non-embryotoxicants but substituting the concentrations that causes a reduction of 50% in the differentiation of beating cardiomyocytes by either ECD200 or ECD50 estimated as above. Each of the 16 genes yielded an individual prediction (strong, weak or non-embryotoxicant) and we took

as global prediction the category with the highest number of individual predictions. Our procedure yielded a 100% of concordance between in vitro global predictions and in vivo outcome of the 7 model chemicals.

Thus, our methodology was reliable, faster and with higher technical simplicity than the classical EST and with the additional advantage of using a wide array of biomarker genes that allows screen embryotoxicants with different mechanisms of action. In addition, this approach might be enlarged testing a larger battery of biomarker genes that yielded a higher number of individual predictions, which would generate safer predictions.

See detailed information about this procedure in the following publication: Romero AC, del Rio E, Vilanova E, Sogorb MA (2015) RNA transcripts for the quantification of differentiation allow marked improvements in the performance of embryonic stem cell test (EST). Toxicology Letters 238: 60-69.
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