

PROGRESS WITH MILAGRO DE LA SELVA (DBT)*

A. Cytotoxicity Studies

I. On Liver Cells- HepG2

Test: Cytotoxicity studies were done on human liver cell line, HepG2 by MTT assay.
Result: Cells were able to tolerate up to 7000ug/ml of DBT dissolved in water.

II. On Fibroblasts (Adipocytes)- 3T3L1

Test: Cytotoxicity studies were done on mouse adipocytes, 3T3-L I by MIT assay.
Result: Cells were able to tolerate up to 200 ug/ml of DBT dissolved in water.

III. On Skeletal Muscle - C2C12

Test: Cytotoxicity studies were done on human liver cell line, HepG2 by MIT assay.
Result Cells were able to tolerate up to 200 ug/mJ of DBT dissolved in water.

B. Screening for glycogen synthesis and adipogenesis

I. Glycogen synthesis

Test Ia. : RT-PCR

RT-PCR for glycogen synthase gene was performed in HepG2 (liver) cell line at various concentrations ranging from 1000 ug/ml to 5000 ug/ml. Glycogen Synthase gene was found to be activated at 3000 ug/rnl . .

Test Ib. : Biochemical

DBT was not found to have any effect on glycogen synthesis to human hepatocytes (HepG2) at up to 1000 ug/rnl. Cells did not survive long for biochemical analysis above 1000 ug/ml .

II. Adipogenesis

Test Ba. : RT-PCR

3T3-LI (mouse fibroblast cells) couldn't tolerate high dosage of DBT., Thus experiments were carried out at dosage ranging from 10ug/rnl to 100ug/ml. Fatty acid synthase gene was not found to be activated up to a concentration of 100 ug/ml.:

Test Ib. : Biochemical

DBT was not found to have any effect on adipogenesis of mouse fibroblasts (3T3-LI) at up to 100 ug/ml. Formation of lipid droplets were tested by Oil-Red-O staining and triglyceride content estimation.

***Diabetic Tea**



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C. Oligo Microarray Profiling

Effect of DBT was tested on the following four tissue systems by oligo-microarray in May'06:

- (i) Human Liver- HepG2
- (ii) Mouse skeletal Muscle - C2C12
- (iii) Mouse Adipocytes – 3T3LI
- (iv) Mouse Pancreatic BetaCells- Beta-Tc6

Analysis results are awaited.

