

X Reunião Ibérica

Fitoplâncton Tóxico e Biotoxinas

Lisboa, 12-15 Maio de 2009

Citotoxicidade e alterações citopatológicas induzidas pela MCLR em linhas celulares renais e hepáticas (Vero e HepG2)



Instituto Nacional de Saúde
Doutor Ricardo Jorge

Carina Menezes

Laboratório de Biologia e Ecotoxicologia
Departamento de Saúde Ambiental



FACULDADE DE CIÊNCIAS | UNIVERSIDADE DE LISBOA

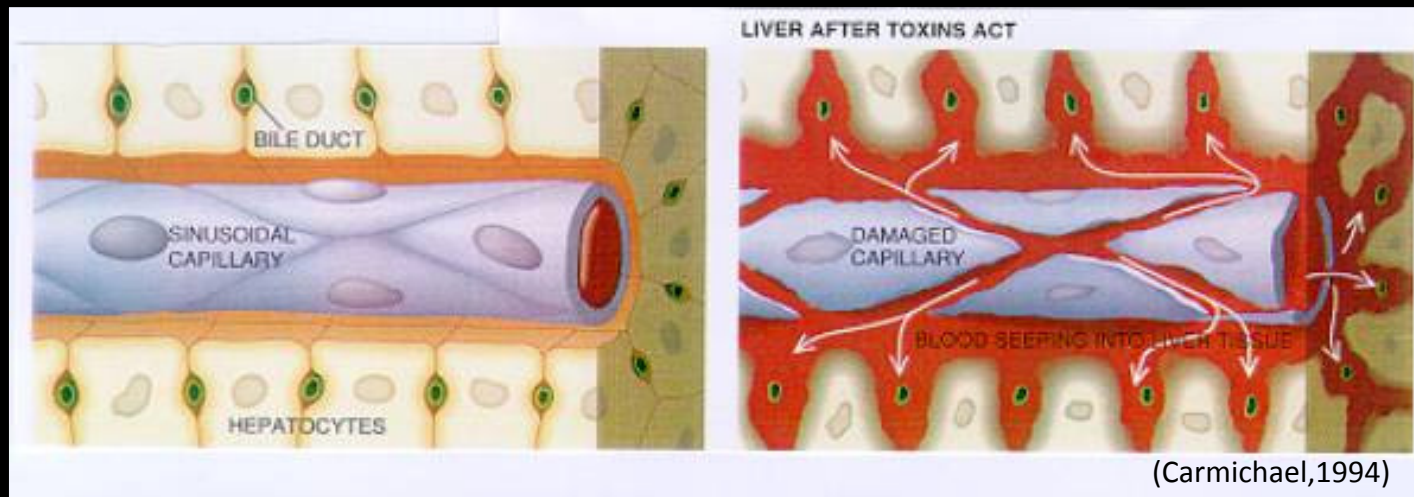
Microcistina-LR

Hepatotoxina → Inibição das fosfatases proteicas 1 e 2A:

Hiperfosforilação de
proteínas do citosqueleto

Perda da
integridade celular

Hemorragia
hepática



Microcistina-LR

Dias *et al*, 2009
Alverca *et al*, 2009

Células Vero

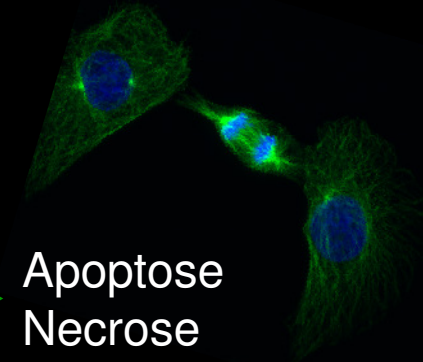
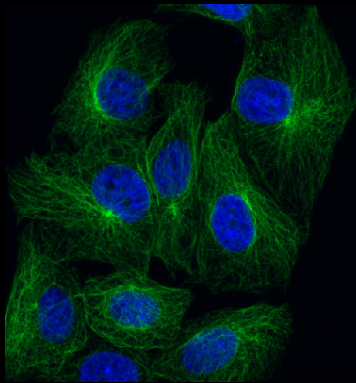
Doses altas de MCLR

Apoptose
Necrose

Doses baixas de MCLR

Autofagia

RE e lisossomas



Mecanismo celular de resposta à toxina?

Ocorre em hepatócitos?



Objetivos

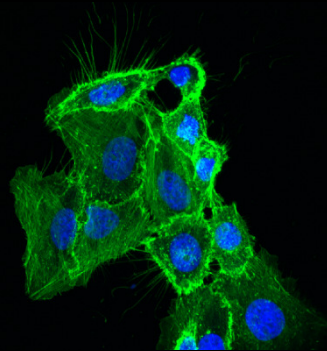
1. Determinação do limiar de citotoxicidade

Teste do Vermelho Neutro

Teste do MTT



2. Determinação dos efeitos da MCLR na morfologia e ultraestrutura celular

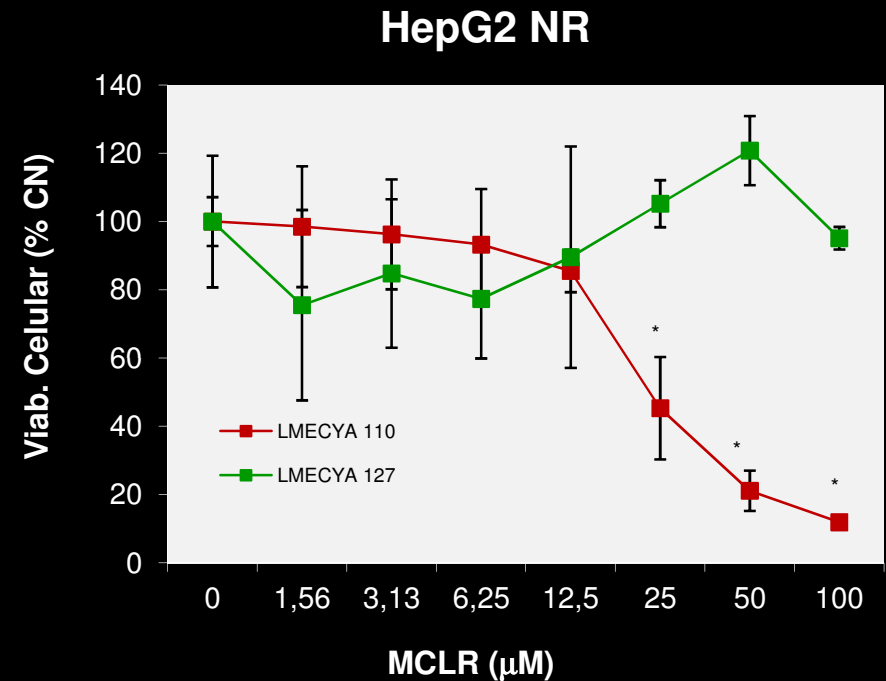
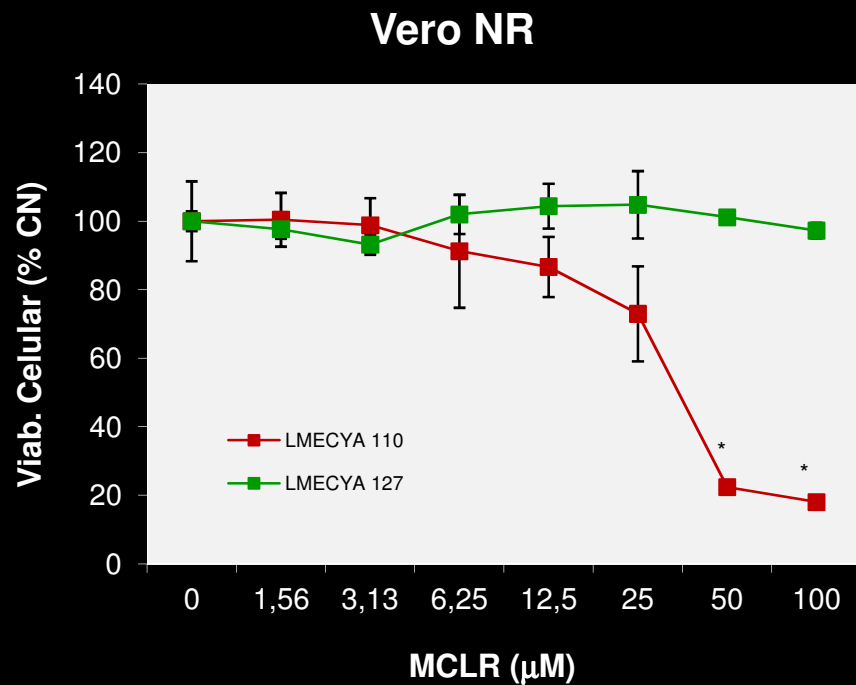


Imunofluorescência e marcação específica:

- ▶ α -LC3B – Autofagossomas
- ▶ α -GRP94 – Retículo Endoplasmático
- ▶ Laranja de Acridine (AO) – Lisossomas
- ▶ Rodamina-123 - Mitocôndrias

Microscopia electrónica de transmissão

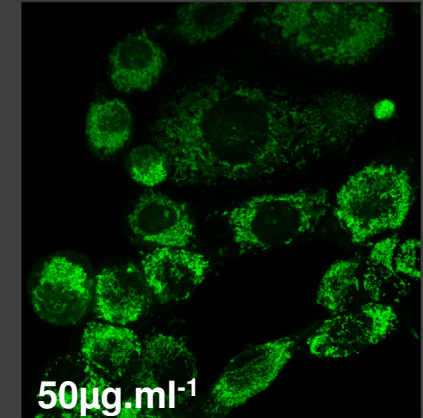
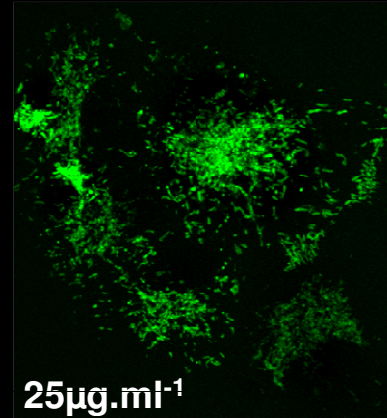
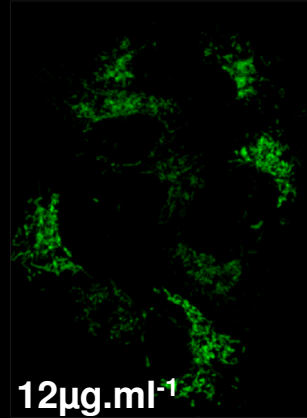
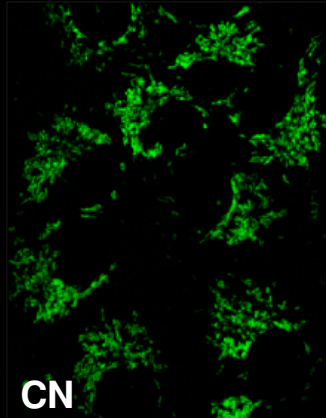
Viabilidade Celular



A diminuição da viabilidade celular é dependente da [MCLR].

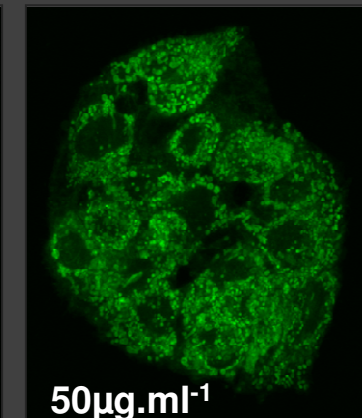
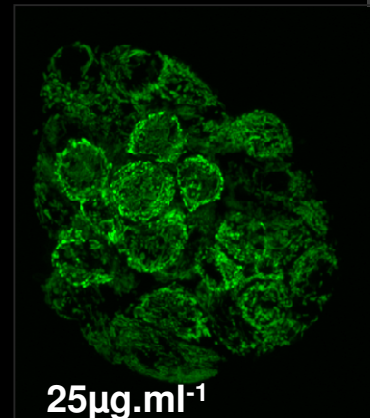
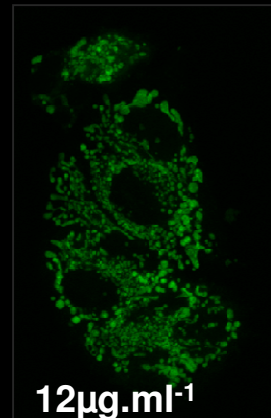
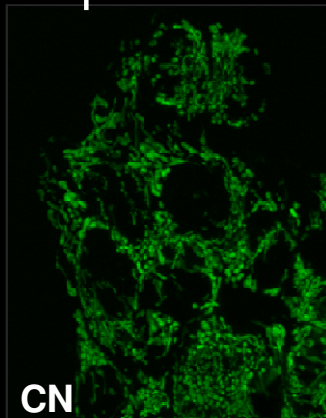
Mitocôndrias (Rh-123)

Vero



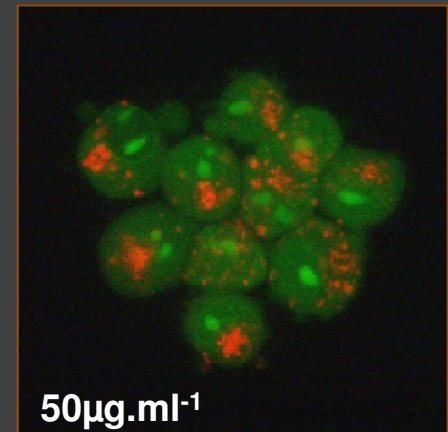
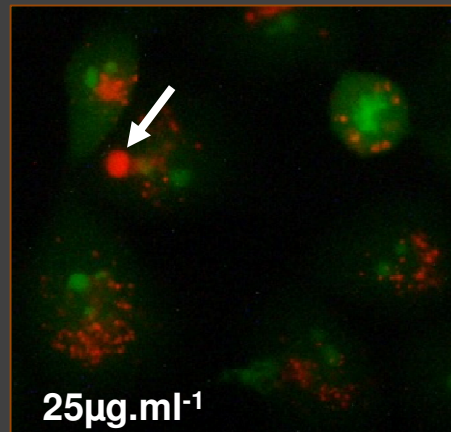
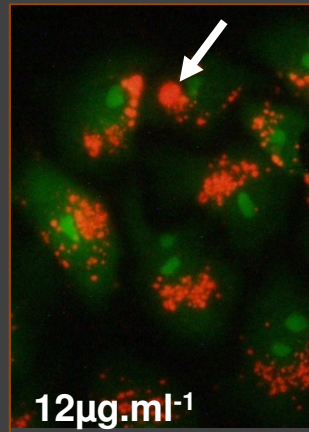
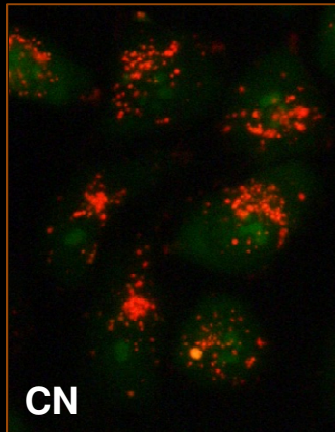
Ruptura mitocondrial

HepG2



Lisossomas (AO)

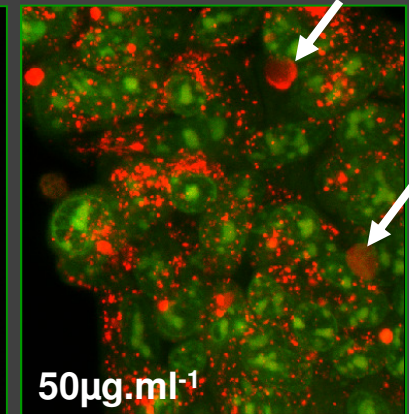
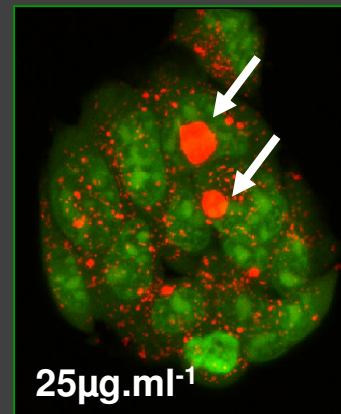
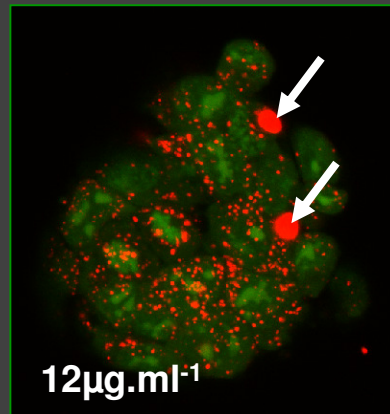
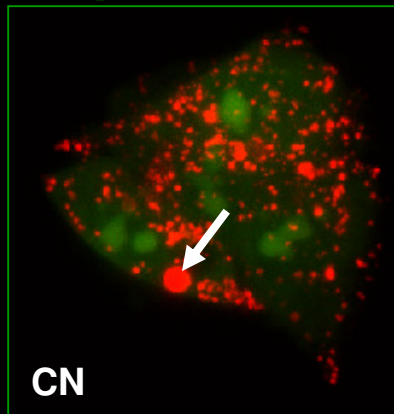
Vero



Diminuição do número de lisossomas

Ruptura lisossomal

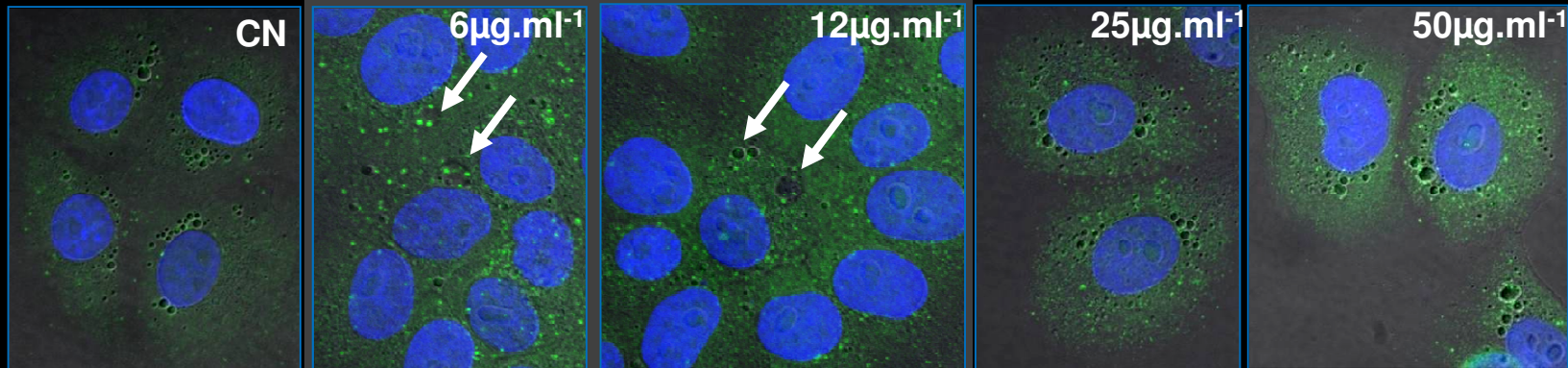
HepG2



Aumento do tamanho dos lisossomas

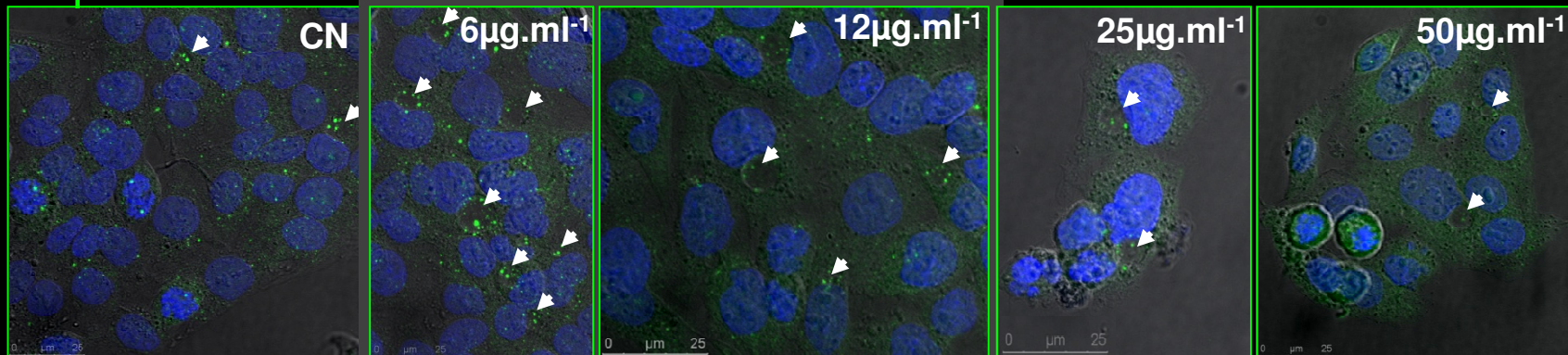
Autofagossomas (LC3B)

Vero



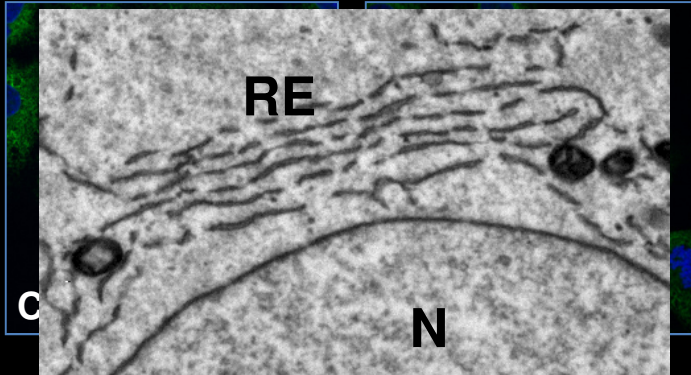
Estimulação da autofagia

HepG2

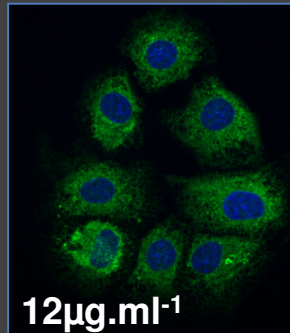


Retículo Endoplasmático (GRP94)

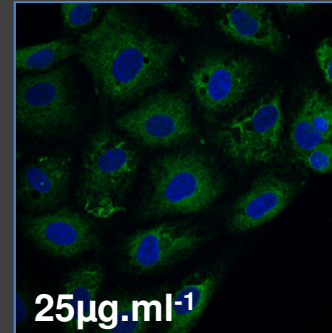
Vero



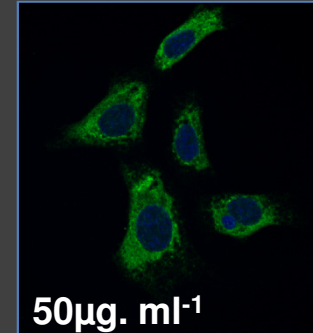
Alverca *et al*, 2009



12µg.ml⁻¹



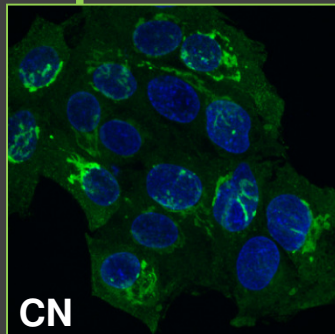
25µg.ml⁻¹



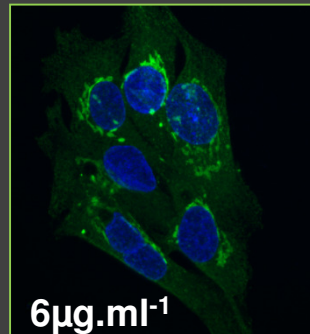
50µg. ml⁻¹

Redistribuição de GRP94: padrão disperso para perinuclear

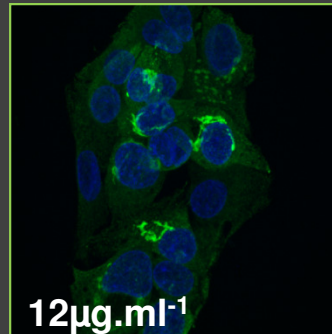
HepG2



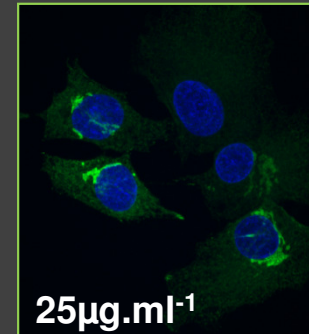
CN



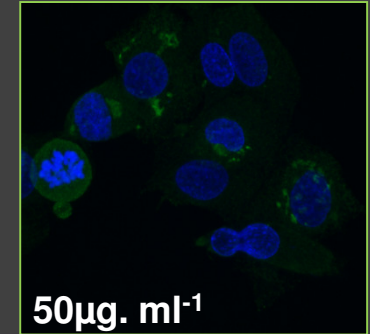
6µg.ml⁻¹



12µg.ml⁻¹



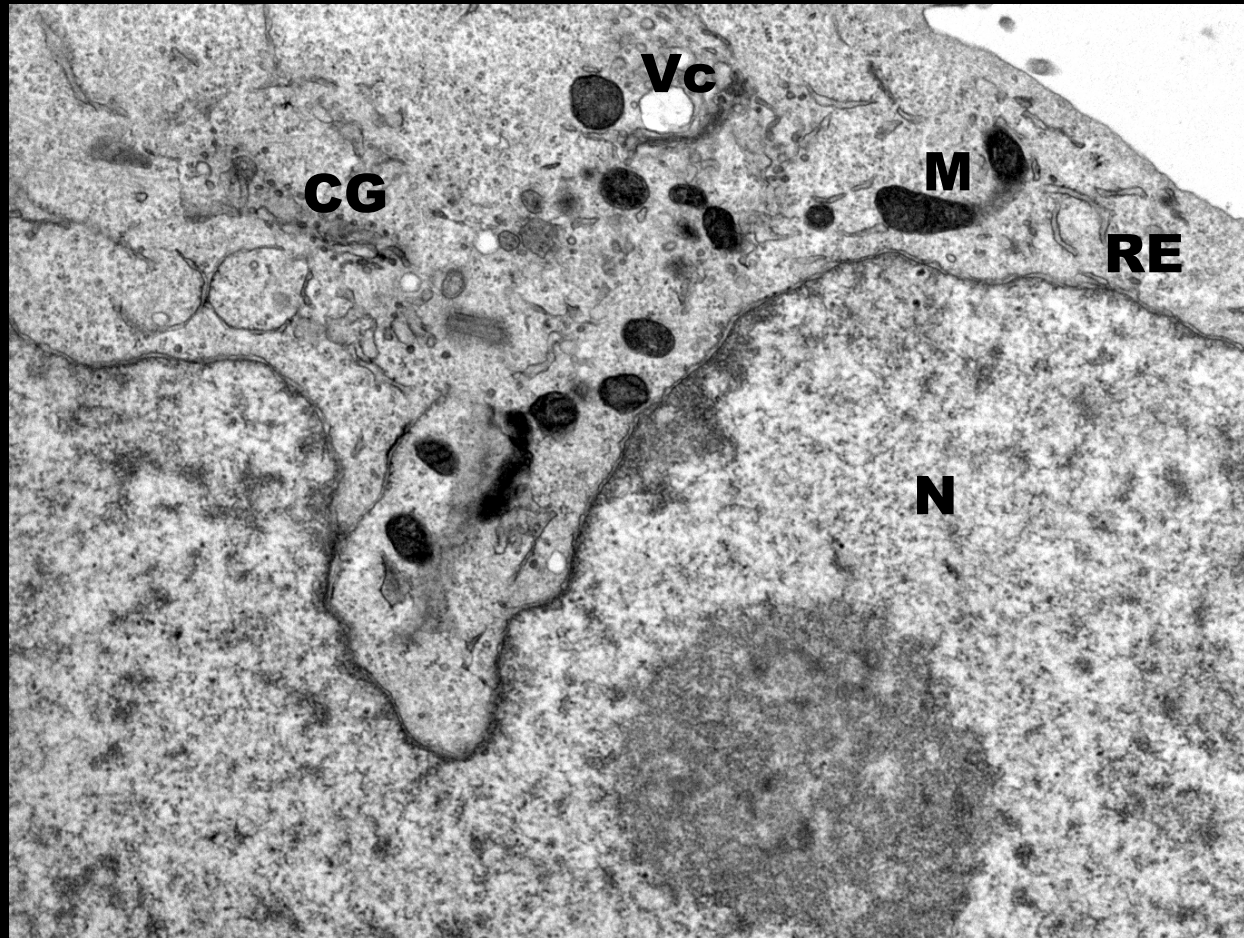
25µg.ml⁻¹



50µg. ml⁻¹

Diminuição da expressão da proteína GRP94

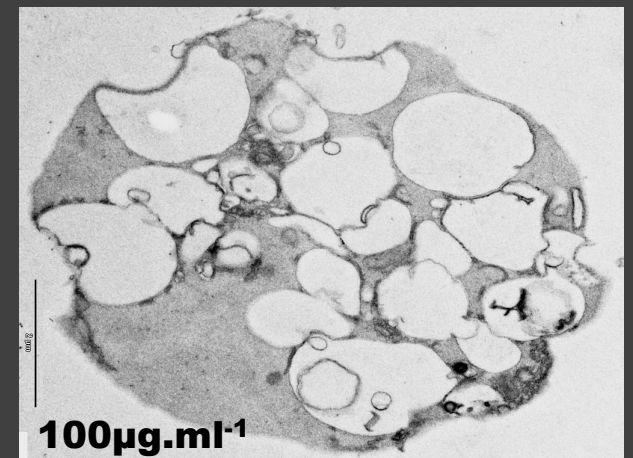
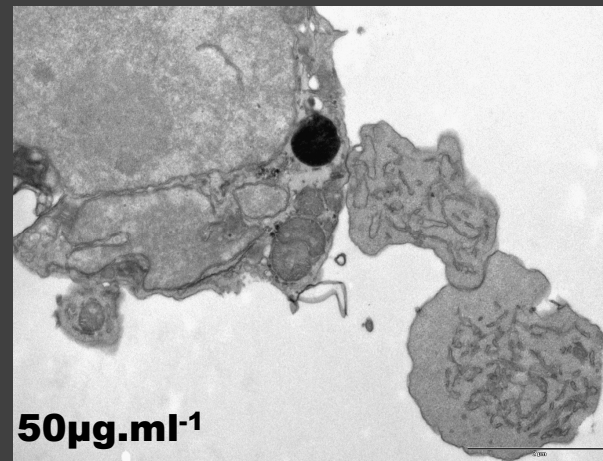
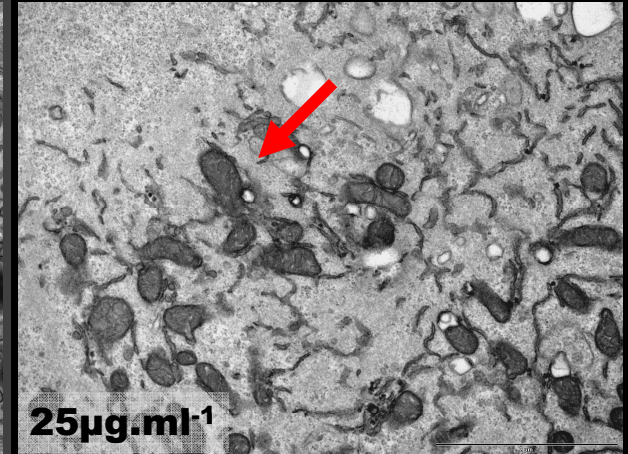
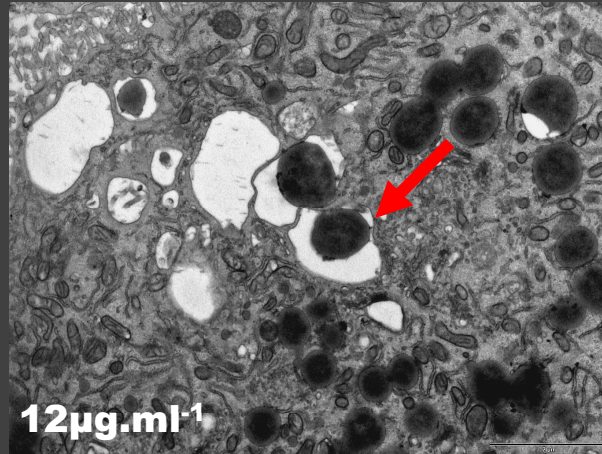
Ultraestrutura de células Vero



Aspecto de células Vero não expostas à toxina

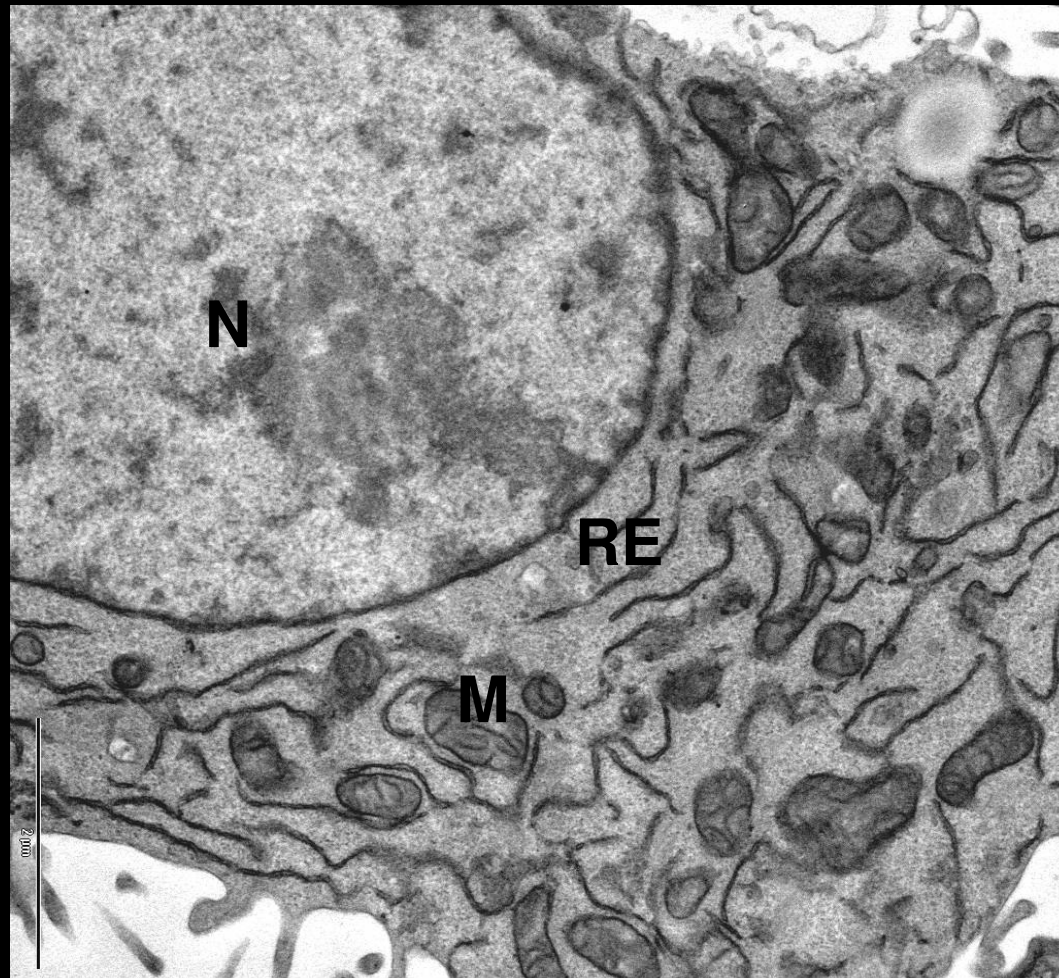
Ultraestrutura de células Vero

Autofagia



Apoptose

Ultraestrutura de células HepG2

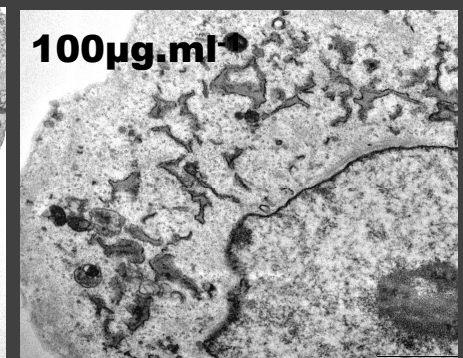
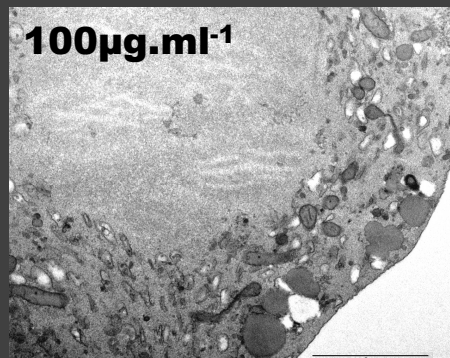
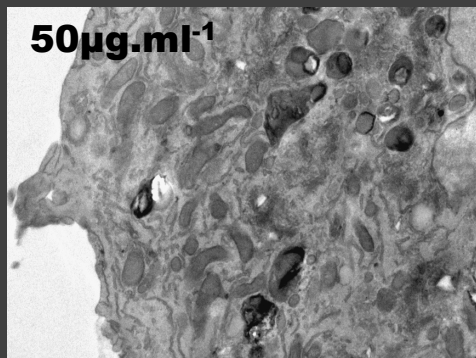
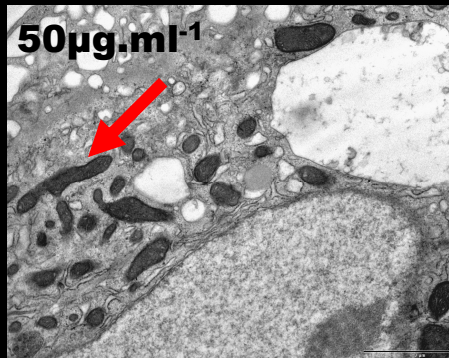
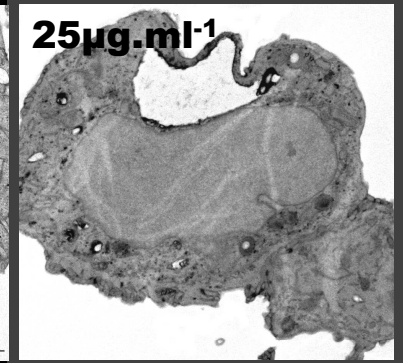
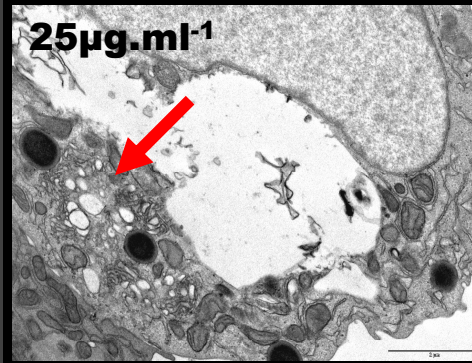
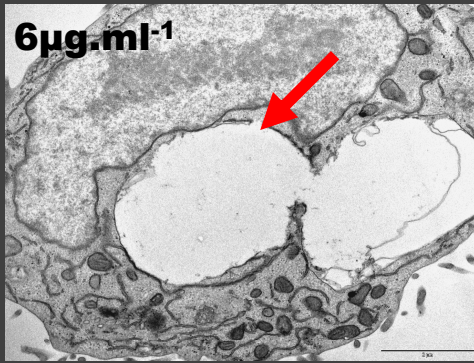


Aspecto de células HepG2 não expostas à toxina

Ultraestrutura de células HepG2

Autofagia

Apoptose



Apoptose

Apoptose

Necrose

Conclusões

- A MCLR induz a perda da viabilidade celular em ambas as linhas celulares
- A MCLR afecta vários organelos intracelulares
- A autofagia é comum a ambas as linhas celulares (a doses mais baixas de toxina)

Efeito diferencial da MCLR na proteína anti-apoptótica GRP94:

↓ Expressão da GRP94 em células HepG2 → ↑ Apoptose → > Sensibilidade das células hepáticas



Agradecimentos

Elsa Dias
Sérgio Paulino
Filomena Sam-Bento
Paulo Pereira
Maria João Silva
Paulo Matos
Ana Amorim
Elsa Alverca



Instituto Nacional de Saúde
Doutor Ricardo Jorge

HABCOL-PROJECTO Nº PDCT/MAR/60086/2004