Review of experimental models for inducing hepatic cirrhosis by bile duct ligation and carbon tetrachloride injection

Revisão de modelos experimentais de cirrose hepática induzida por ligadura do ducto biliar e por injeção de tetracloreto de carbono

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ABSTRACT
PURPOSE: To present a review about a comparative study of bile duct ligation versus carbon tetrachloride injection for inducing experimental liver cirrhosis.

METHODS: This research was made through Medline/PubMed and SciELO web sites looking for papers on the content “induction of liver cirrhosis in rats”. We have found 107 articles but only 30 were selected from 2004 to 2011.

RESULTS: The most common methods used for inducing liver cirrhosis in the rat were administration of carbon tetrachloride (CCl4) and bile duct ligation (BDL). CCl4 has induced cirrhosis from 36 hours to 18 weeks after injection and BDL from seven days to four weeks after surgery.

CONCLUSION: For a safer inducing cirrhosis method BDL is better than CCl4 because of the absence of toxicity for researchers and shorter time for achieving it.

Key words: Liver Cirrhosis. Drugs. Carbon Tetrachloride. Common Bile Duct. Rats.

RESUMO
OBJETIVO: Apresentar revisão sobre estudo comparativo da indução de cirrose hepática (CH) experimental com a injeção de tetracloreto de carbono (CCl4) comparado à ligadura do ducto biliar (BDL).

MÉTODOS: A pesquisa foi realizada nas bases de dados do Medline/PubMed e SciELO procurando trabalhos com as palavras indução de CH e ratos. Foram encontrados 107 artigos, mas somente 30 foram selecionados no período de 2004 à 2011.

RESULTADOS: Os procedimentos mais comum para indução de CH em ratos foram a injeção de CCl4 e a BDL. O CCl4 induziu CH no período de 36 horas após a injeção e a DBL de sete dias à quatro semanas após a cirurgia.

CONCLUSÃO: A BDL é o método mais seguro para indução de CH quando comparado a injeção de CCl4 pela ausência de toxicidade para os pesquisadores e o menor tempo para se obter a lesão hepática.

Introduction

Liver cirrhosis (LC) is considered a public health, according to World Health Organization, about 800 thousand people die from LC every year. Only in United States LC is responsible for around 27 thousand deaths per year, representing a mortality rate of 9.2 per 100,000, placing it as the 12th overall cause of death1-3. Nowadays major research centers focus on studying LC, its mechanisms and its behavior, complications and possible treatments. For that reason it has been developed, efficient experimental models of induction of LC in rats. The two most common methods used for experimental LC are the administration of carbon tetrachloride (CCl4) and the bile duct ligature (BDL)7-9. Our aim is to present a comparative study of bile duct ligature versus carbon tetrachloride injection for inducing experimental liver cirrhosis.

Methods

This research was made through Medline/PubMed and SciELO web sites looking for papers on the content “induction of liver cirrhosis in rats”. We found 107 articles but only 30 from 2004 to 2011 were selected. The inclusion criteria were: only rats; bile duct ligature and injection of carbon tetrachloride. Histopathologic examination for confirming cirrhosis; Time of inducing cirrhosis. The exclusion criteria were: large animals cirrhosis; others rodent animals; drug inducing cirrhosis such as dimethylnitrosamine; thioacetamide; butylhydroperoxide. Others drugs association methods: buprenorphine with reduction of portal inflow over a stent inserted in the right renal artery; Others methods: unrestricted flow using an aortic-portal segment; orthotopic liver transplantation with unrestricted portal arterialisation. Thioacetamide associated with partial hepectomy.

Results

The main methods of induction of LC in rats are the administration of carbon tetrachloride and bile duct ligature. Below are shown induction of cirrhosis by CCl4, dosage of the drug, time for inducing cirrhosis, main test assessment with the method and their results (Table 1) and for bile duct ligature (Table 2).
### Table 1: Induction of cirrhosis in rats by carbon tetrachloride (CCl4)

<table>
<thead>
<tr>
<th>Author(s) / Year</th>
<th>Dosage of CCl4</th>
<th>Number of Rats</th>
<th>Time to Induce Cirrhosis</th>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maya-Mendoza et al., 2004</td>
<td>ND</td>
<td>ND</td>
<td>8 weeks</td>
<td>Explore genes with differential activity or position in the nuclear matrix</td>
<td>Changes in the relative position of specific genes to the nuclear matrix occurring during the chronic administration of CCl4</td>
</tr>
<tr>
<td>Feng et al., 2003</td>
<td>ND</td>
<td>60</td>
<td>ND</td>
<td>Expression of bFGF and HGF</td>
<td>FGFR regulates liver fibrogenesis through regulating metabolism of extracellular matrix</td>
</tr>
<tr>
<td>Ficuci et al., 2006</td>
<td>300 mg/kg/100 g</td>
<td>22</td>
<td>4 weeks</td>
<td>Farnesoid X receptor</td>
<td>PPAR promotes the development of a quiescent phenotype and increases apoptosis of HSCs</td>
</tr>
<tr>
<td>Lewis et al., 2005</td>
<td>1.5 mL/kg</td>
<td>8</td>
<td>16 hours</td>
<td>CYP2E1</td>
<td>The CYP2E1 can induce endoplasmic reticulum protein damage and stress via its catalytic activation of pro-oxidants</td>
</tr>
<tr>
<td>Oja et al., 2006</td>
<td>200 mg/kg/week</td>
<td>41</td>
<td>8 – 14 weeks</td>
<td>Propofol</td>
<td>Reproduce functional abnormalities of the central motor tract</td>
</tr>
<tr>
<td>Kostolou et al., 2006</td>
<td>ND</td>
<td>40</td>
<td>6 weeks</td>
<td>GS-propranolol; G2-propranolol + lidocaine; GS-Propranolol + CCl4; G4-propranolol + lidocaine + CCl4</td>
<td>Propranolol dosage should be reduced when lidocaine is co-administered</td>
</tr>
<tr>
<td>Li et al., 2005</td>
<td>2 mg/kg</td>
<td>ND</td>
<td>6 weeks</td>
<td>Small interfering RNA</td>
<td>Prevent liver fibrosis</td>
</tr>
<tr>
<td>Tsut et al., 2006</td>
<td>0.2 mL/kg/week</td>
<td>ND</td>
<td>9 weeks</td>
<td>Heme oxygenase</td>
<td>Suppresses the development of cirrhosis</td>
</tr>
<tr>
<td>Ohgura et al., 2006</td>
<td>ND</td>
<td>42</td>
<td>8 weeks</td>
<td>Wnt1</td>
<td>Reduces liver fibrosis and improves liver function</td>
</tr>
<tr>
<td>Liu et al., 2006</td>
<td>1.5 mL/kg in liquid paraffin; 1x twice a week for 8 weeks</td>
<td>70</td>
<td>8 weeks</td>
<td>Ginag Pigeon Et al.</td>
<td>Inhibits the HSC</td>
</tr>
<tr>
<td>Xue et al., 2007</td>
<td>5 or 3 mL/kg in 40 mL olive oil twice a week</td>
<td>ND</td>
<td>11 weeks</td>
<td>Hemin</td>
<td>Liver protection</td>
</tr>
<tr>
<td>Shen et al., 2008</td>
<td>0.4 mL/kg in corn oil; 1.5 once time</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Increases of Smad expression</td>
</tr>
<tr>
<td>Ali et al., 2008</td>
<td>3 mL/kg twice a week</td>
<td>ND</td>
<td>7 weeks</td>
<td>Deltaferin sodium</td>
<td>Enhances hepatic regeneration and minimizes hepatic fibrogenesis</td>
</tr>
<tr>
<td>Yuan et al., 2008</td>
<td>50% in Olive Oil</td>
<td>40</td>
<td>18 weeks</td>
<td>Bodanskis lipoprotein</td>
<td>Decrease hepatic desease</td>
</tr>
<tr>
<td>Donaldson-Kempster et al., 2006</td>
<td>2 mL/kg in mineral oil; same volume</td>
<td>20</td>
<td>12 weeks</td>
<td>ND</td>
<td>PDGF expression is related to liver regeneration</td>
</tr>
<tr>
<td>Tiberio et al., 2008</td>
<td>0.02 mL/g 300 g once a week</td>
<td>ND</td>
<td>15 weeks</td>
<td>IL-6</td>
<td>Liver regeneration</td>
</tr>
<tr>
<td>Tan et al., 2008</td>
<td>3.5 mL/kg twice a week in corn oil; 1.5</td>
<td>20</td>
<td>8 weeks</td>
<td>Siloamin</td>
<td>Regeneration (decrease AST, ALT, FA)</td>
</tr>
<tr>
<td>Kim et al., 2008</td>
<td>ND</td>
<td>2 weeks</td>
<td>Retinane</td>
<td>Decrease cirrhosis</td>
<td></td>
</tr>
</tbody>
</table>

**ND** - Not Described; **CCl4** - Carbon Tetrachloride; **bFGF** - Basic Fibroblast Growth Factor; **FXR** - Farnesoid X receptor; **HGF** - Hepatocyte growth factor; **HSC** - Hepatic stellate cell; **CYP2E1** - Cytochrome P450 2E1; **Smad1** - Gene Mothers Against Decapentaplegic 1; **PDGF** - Platelet-Derived Growth Factor; **IL-6** - Interleukin 6; **AST** - Aspartate Transaminase; **ALT** - Alanine Transaminase; **FA** - Phosphotase Alkaline.

### Table 2: Induction of cirrhosis in rats by using bile duct ligation (BDL)

<table>
<thead>
<tr>
<th>Author(s) / Year</th>
<th>Number of rats</th>
<th>Time to Induce Cirrhosis</th>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antoine et al., 2005</td>
<td>ND</td>
<td>2 weeks</td>
<td>Phenabrain</td>
<td>Phenabrain will increase the HSC expression</td>
</tr>
<tr>
<td>Huang et al., 2006</td>
<td>ND</td>
<td>5 weeks</td>
<td>Tet and zylparin</td>
<td>They reduced the fibrosis scores and hepatic collagen content of BDL rats</td>
</tr>
<tr>
<td>Son et al., 2009</td>
<td>68</td>
<td>5 weeks</td>
<td>Bacterial translocation</td>
<td>Bacterial translocation has a role in the pathogenesis of hepatic pulmonary syndrome by inducing pulmonary intravascular macrophages through TNF-alpha upregulation</td>
</tr>
<tr>
<td>Feuer et al., 2007</td>
<td>40</td>
<td>2 weeks</td>
<td>Phospholipid before or after sham operation or BDL</td>
<td>Lowered hepatic iron concentration. After BDL, body weight increase, lower hepatic weight, less portal hypertension, less periportal necrosis, less portal inflammation, lower hepatic activity index score and higher albumin levels</td>
</tr>
<tr>
<td>Aman et al., 2008</td>
<td>ND</td>
<td>7 days</td>
<td>Bortezomib and MD1331</td>
<td>Inducing HSC apoptosis and inhibiting liver fibrogenesis</td>
</tr>
<tr>
<td>Makemi et al., 2007</td>
<td>4 weeks</td>
<td>E-cadherin, zinc content, and zinc-dosage</td>
<td>Protected portal hypertensive gastric mucosa with increased HGF2 expression</td>
<td></td>
</tr>
<tr>
<td>Tizzolo et al., 2007</td>
<td>28</td>
<td>28 days</td>
<td>Quecetin</td>
<td>Quercetin treated cirrhotic rats showed reduced DNA damage in lung and liver tissues as compared to untreated cirrhotic rats</td>
</tr>
<tr>
<td>Lee et al., 2007</td>
<td>ND</td>
<td>27 days</td>
<td>YPGF (Yin-Shen-Mao-Tang)</td>
<td>Hepatic hypoxemia accumulation and hepatic collagen levels can be decreased</td>
</tr>
<tr>
<td>Thomsen et al., 2008</td>
<td>ND</td>
<td>1 month</td>
<td>LPS + IFN-2 (infection simulation)</td>
<td>Accelerated tissue loss during infection</td>
</tr>
<tr>
<td>Langer et al., 2008</td>
<td>ND</td>
<td>4 weeks</td>
<td>ND</td>
<td>Nitric oxide induce HSC apoptosis</td>
</tr>
<tr>
<td>Vellatino et al., 2008</td>
<td>24</td>
<td>2 weeks</td>
<td>N-Acetyl cysteine</td>
<td>Protective effects in cirrhotic rats with hepatoportal syndrome</td>
</tr>
<tr>
<td>Hagens et al., 2008</td>
<td>ND</td>
<td>30 days, 3 weeks</td>
<td>ND</td>
<td>A new HSC type was found</td>
</tr>
</tbody>
</table>

**BDL** - Bile Duct Ligation; **HGF2** - 2-HO Acetaminophen Protein; **HSC** - Hepatic Stellate Cells; **LPS** - Lipopolysaccharide; **HGF-1** - Insulin-like Growth Factor 1.
Marques TG et al.

Discussion

The main methods of induction of LC in rats are the administration of carbon tetrachloride (CCl4) and bile duct ligation (BDL). CCl4 is one of the most used methods nowadays however it is considered an extremely toxic method. Several important basic mechanisms of tissue damages induced by CCl4 have emerged, involving metabolic activation, reactive free radical metabolites, lipid peroxidation, covalent binding and disturbance of calcium homeostasis.

The CCl4 administration results in hepatocyte damage, necrosis, inflammation, and fibrosis, which spreads to link the vascular structures that feed into and drain the hepatic sinusoid (the portal tract and central vein radicle, respectively). It activates the hepatic stellate cell (HSC) inducing hepatocyte apoptosis and zone III necrosis. Continuous administration of CCl4 can provide moderate cell necrosis and fatty infiltration in four weeks.

The CCl4 is excreted from the body within the first 24 hour by conjugation reaction mediated by phase. In the literature, three mechanisms have been proposed as the possible explanations for progression of injury: (1) Contribution of inflammatory cells; (2) Production of free radicals; and (3) Leakage of degradative enzymes from the dying and injured cells. Activated resident Kupffer cells and the neutrophils recruited at the site of parenchymal liver injury are considered as the primary culprits in damaging surrounding healthy cells as the result of nonspecific action. However, evidence suggests that the contribution of the inflammatory cells does not or is not sufficient to mediate progression of injury.

The second theory regarding progression of injury is production of free radicals and oxidative stress, and subsequent lipid peroxidation that propagates injury. Though the antioxidants prevent/delay the tissue damage partially, progression of injury still occurs. The inhibition of lipid peroxidation by antioxidants only decreases the initial injury of CCl4, blocking lipid peroxidation occurs. The inhibition of lipid peroxidation by antioxidants only prevents progression of injury and subsequent lethality. By 8 weeks, a micronodular cirrhosis takes place. It has been used mainly by intraperitoneal injection or oral administration, the dosage 0.2 to 5ml/kg and LC is achieved between 36 hours to 18 weeks.

BDL is a safer method comparing to the CCl4. When the BDL is done, it provides an acute obstructive jaundice in two weeks, and progression to cirrhosis in 4 or 6 weeks. BDL stimulates the proliferation of biliary epithelial cells and oval cells (which are hepatocyte progenitors), resulting in proliferating bile ductules with an accompanying portal inflammation and fibrosis.

Cholangiocyte proliferation started after BDL at the edge of the portal tract. During the first week from BDL the hepatic microcirculation did not show any alterations with respect to the normal liver. Using this method LC is achieved between seven days to four weeks.

Conclusions

Based on our broadly review we concluded that LC can be induced by CCl4 and BDL, however the manipulation of CCl4 can be dangerous for the researcher with risk of inducing liver tumor. BDL is a safer method than CCl4 and cirrhosis could be induced in rats in average mean time of two weeks.

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