Expression of cytokeratin – 7 and cytokeratin – 19 in mice model biliary atresia

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ABSTRACT

Background: Biliary atresia (BA) is a progressive inflammatory obstruction and fibro-obliteration of the partial or total extrahepatic and intrahepatic bile ducts during the perinatal period, with the etiology remains uncertain. In addition to damage caused by an immunological response, bile duct epithelial cells is thought to occur changes in the characteristics of epithelial cells into mesenchymal cell characteristics, that known as Epithelial Mesenchymal Transition (EMT). In the process of EMT, expression of cytokeratin – 7 (CK-7), cytokeratin – 19 (CK-19) is decreased. Objective: to investigate the changes of CK-7 and CK-19 expression in mice models Biliary Atresia. Method: Two groups of neonatal mice, those were trial group consisted of neonatal mice injected by RRV 1.5 x 10^6 PFU intraperitoneally less than 24 hours after birth, and control group consisted of neonatal mice injected with buffered saline were used as BA model. The expressions of CK-7 and CK-19 of the two groups of neonatal mice were studied on day 3rd, 7th, 14th, and 21st after birth by flow cytometry examination. Results: Median value of CK-7 and CK-19 expressions in trial group on day 3rd, 7th, 14th, and 21st significantly lower than control group (p<0.05). The difference in the value of CK-7 expression between trial group and control group progressively seen from day 7th and the large difference in expression values obtained on day 21st. Expression of CK-19 of trial group increased after decreased on day 3rd and continued until day 21st although it remained below the pattern control group. Conclusion: The greatest decreased of CK-7 expression in biliary atresia mice model was obtained on day 21st after induction of RRV, while the expression of CK-19 still increased.

Original Article

1. Introduction

Biliary atresia (BA) is a progressive inflammatory obstruction and fibro-obliteration of the partial or total extrahepatic and intrahepatic bile ducts during the perinatal period. 1,2,3 Biliary atresia occurs in about 1 in 5000 to 8000 live births, and 50% require liver transplantation. 4,5 In Soetomo hospital Surabaya since 1993-2003 there were 9 patients (9.4%) of 98 patients with neonatal cholestasis. 6

Biliary atresia’s etiology remains uncertain. There are several factors thought to play a role in the pathogenesis of biliary atresia BA include infection, fetal circulatory disorders, abnormal morphogenesis, exposure to toxins, and immunological disorders. 4,7,8 In addition to damage caused by an immunological response, bile duct epithelial cells is thought to occur changes in the characteristics of epithelial cells into mesenchymal cell characteristics. Bile duct epithelial cells lose cell polarity, cell communication, loss of normal structure of epithelial cells accompanied by the accumulation of extracellular matrix (MES). 9,10,11 This process is known as Epithelial Mesenchymal Transition (EMT). 12,13,14,15

In the process of EMT is characterized by decreased expression of calcium-dependent adhesion Epithelial (E-cadherin), cytokeratin – 7 (CK-7), cytokeratin – 19 (CK-19), integrins and increased expression of calcium-dependent adhesion Neural (N-cadherin), Vimentin, Fibronectin, α - Smooth Muscle Actin (α-SMA), Fibroblast Specific protein-1 (FSP-1). 16,17,18 Studies in cell culture defined decreasing of epithelial cells markers such as cytokeratins and increasing of mesenchymal cells markers. 19,20,21

The purpose of this study is to investigate the changes of CK-7 and CK-19 expression in mice models Biliary Atresia.
MATERIALS AND METHODS

Biliary atresia mice model

Twenty timed pregnant female BALB/c mice were kept in micro isolator cages in a virus-free environment at Molecular Biology Laboratory of the Faculty of Medicine Brawijaya University Malang Indonesia. They had free access to sterilized chow and water. Forty-eight babies of mice were given a single intraperitoneal (i.p.) injection of The RRV strain MMU 18 006 (1.5 X 10^6 pfu/mL) or balanced salt solution (BSS) as a control within 24 hours of life. Infected babies of mice that died within the first 2 days, or were not fed by their mothers after infection, were excluded from further analysis and counted as early lethality. Weight of babies of mice was determined at day 3rd, 7th, 14th, and 21th of life. Babies of mice were sacrificed on day 3rd, 7th, 14th, and 21th of life from each group. Liver and biliary tissues were removed at sacrifice and processed for flowcytometry. All efforts were made to minimize babies of mice suffering. Animal sacrifice was performed by cervical dislocation. The Ethical Committee of Health Research Faculty of Medicine Brawijaya University Malang Indonesia approved all animal protocols.

Flowcytometric analysis

Tissue was homogenized and red cells lysed with ACK buffer. Liver immune cells were enriched by Percoll gradient (40/60). Single-cell suspensions were incubated with Fc-block and ready for stained. A mouse CK-7 and CK-19 staining kits was used according to the manufacturer’s instructions (eBioscience, San Diego, CA). Cells were visualized with FACS Caliber flow cytometer (Becton-Dickinson, Mountain View, CA), FlowJo (Tree Star, Inc., Ashland, OR) software used for analysis. Flowcytometric analysis was done at Biomedical Laboratory the Brawijaya University Malang Indonesia.

Statistical analysis

Data were analyzed statistically using IBM SPSS 20 software on personal computer. Descriptive analysis was used to describe the characteristics of the study subject. Analysis of the average for numerical data used independent sample t-test, Mann Whitney test, and Kruskal-Wallis. Data were analyzed using 95% confidence level ($\alpha = 0.05$).

RESULTS

Totally 48 newborn mice/babies were eligible in this study, consisted of two groups, those were 24 infected by RRV 1.5 x 10^6 PFU intraperitoneally less than 24 hours after birth as study group and the others 24 injected with buffered saline as control group.

Median value of CK-7 expression in trial group quantitatively lower than control group, which shows the influence of the RRV induced changes in the expression of CK-7 murine model of BA day 3rd, 7th, 14th, and 21th after induction compared to the control (Table 1).

Table 1. Changes in the expression of cytokeratin – 7 in the RRV induction groups and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>Control groups</th>
<th>Trial groups</th>
<th>p* between groups per variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (interquartil)</td>
<td>Median (interquartil)</td>
<td></td>
</tr>
<tr>
<td>CK-7</td>
<td>3rd</td>
<td>4.5 (0.06)%</td>
<td>4.2 (0.05)%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>7th</td>
<td>6.9 (0.15)%</td>
<td>3.9 (0.10)%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>20.8 (0.08)%</td>
<td>10.2 (3.22)%</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>21th</td>
<td>25.2 (0.63)%</td>
<td>7.7 (0.71)%</td>
<td>0.00</td>
</tr>
<tr>
<td>p*</td>
<td></td>
<td>0.000</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Following chart (figure 1) shows the changes of CK-7 expression (median) from time to time in the control groups compare with trial groups. The difference in the value of CK-7 expression between trial group and control group progressively seen from day 7th and the large difference in expression values obtained on day 21st.

Figure 1. Effect of induction of RRV and duration of illness after RRV exposure to changes in the expression of cytokeratin – 7 (median) compared to the control groups.

Table 2. Changes in the expression of cytokeratin – 19 in the RRV induction groups and control groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day</th>
<th>Control groups</th>
<th>Trial groups</th>
<th>p* between groups per variables</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (interquartil)</td>
<td>Median (interquartil)</td>
<td></td>
</tr>
<tr>
<td>CK-19</td>
<td>3rd</td>
<td>6.4 (0.64)%</td>
<td>5.3 (0.66)%</td>
<td>0.007**</td>
</tr>
<tr>
<td></td>
<td>7th</td>
<td>18.2 (0.14)%</td>
<td>4.3 (0.09)%</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>14th</td>
<td>19.4 (1.02)%</td>
<td>8.6 (1.59)%</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>21th</td>
<td>23.3 (0.06)%</td>
<td>15.7 (0.71)%</td>
<td>0.000*</td>
</tr>
<tr>
<td>p*</td>
<td></td>
<td>&lt;0.000**</td>
<td>0.002**</td>
<td></td>
</tr>
</tbody>
</table>

*significant at p<0.05.
The following chart (figure 2) shows the expression of CK-19 (median) from time to time in the control groups compared with trial groups. In trial group, expression of CK-19 decreased on day 3rd and then increased until day 21st although it remained below the pattern control group. The difference in the value of CK-19 expression progressively between trial group and control group seen from 3rd and the large difference in expression values obtained on day 3rd.

![CK-19 Chart](image)

Figure 6. Effect of induction of RRV and duration of illness after RRV exposure to changes in the expression of cytokeratin – 19 (median) compared to the control groups.

**DISCUSSION**

This study is an experimental study with Balb/c mice which proves that RRV induction dose of 1.5 x 10^6 PFU intraperitoneally at the age of less than 1 day after birth provides significant effect on the decrease in the expression of CK-7 when compared with controls and the decrease is going according to the time sequence begins after day 3 post-induction RRV. RRV induction would affect changes in cellular adaptive immune response in biliary tract tissue. In this study, the cellular adaptive immune response that occurs is reflected in the presence of expression of CK-7 and CK-19. The hypothesis that pro-inflammatory cytokines important for the pathogenesis of BA has been tested on mice models induced RRV. Bile duct damage initiated by viral infection which is characterized decrease CK-7 and CK-19.

The results of the study Harada (2009) with the dsRNA analog administration on bile duct epithelial cell cultures have reduced expression of CK-7.20 Valdes (2002) with Wistar rats were induced by TGF-β found that there are differences in the expression of CK-7 significantly between groups of families and groups try although the difference was not too big on day 3rd.

At day 7th after induction, also found decreased expression of CK-7 and expression differences greater than the control group. These results are in accordance with Choi (2009) that found the lowest value CK-7 expression at day 7th after administration of CCl4 intraperitoneally in mice. Increased expression of this cytokeratin according to De Vries (2011) caused the entire cell stress induced EMT that occurs more widely. The results corresponded to Feng (2005) gain on day 21st total obstruction extrahepatic bile duct after induction RRV.

In this study, gain reduction the expression of CK-19 in experimental group (p < 0.001) and the longer the exposure time, the virus was also found reduced expression of CK-19 in the experimental group greater than control group. Harada (2009) found that after administration of the dsRNA analog bile duct epithelial cell culture, a decline in CK-19 and other markers of epithelial cells and an increase in markers of mesenchymal cells, such as FSP-1 and vimentin.

In accordance with the result of this study, Yabushita (2001) found a decrease in the expression of CK-19 since day 3rd in mice induced by RRV. De Vries (2011) also found that difference expression of CK-19 were increased compared to control on day 7th, results this is supported by the increased expression of mesenchymal cell markers such as α-SMA.

Expression of CK-19 of trial group increased after decreased on day 3rd and continued until day 21st although it remained below the pattern control group. This is in accordance with Paku (2005) which found that although there has been a total blockage on histopathology examination, however CK-19 can still be detected on flowcytometry examination.

The EMT process in biliary atresia besides a decline in the expression of CK-7 and CK-19, obtained also decrease other markers of epithelial cells (E-cadherin) and an increase in markers of mesenchymal cells such as N-cadherin, vimentin, FSP-1, α-SMA, fibronectin.

The results of this study provide additional evidence of the truth of the hypothesis that the induction of RRV resulted in changes the expression of CK-7 and CK-19 in the pathogenesis of BA, thus opening discourse to do further studies for new strategies in the management of medically BA. Progressive decrease expression of CK-7 and CK-19 beginning on day 7th with a peak at day 21st shows that the possibility of a good time for medical intervention performed around day 7th and before day 14th, because that the occurrence of BA already irreversible.

**CONCLUSION**

The research approved that either the expression of cytokeratin – 7 and cytokeratin – 19 were altered in biliary atresia mice model with different mode of change. The greatest decreased of CK-7 expression was obtained on day 21st after induction of RRV, while the expression of CK-19 still increased.

**REFERENCES**

5668


