Effect of *in vitro* isolation and starvation on cholesterol content in *Paramphistomum cervi*

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Abstract

The effect of *in vitro* isolation and the starvation on the cholesterol contents was investigated in *Paramphistomum cervi*. The cholesterol contents in the body of the parasite decreased when glucose was available (0.071 Control, 0.070 (30 mins), 0.069 (1 hr), 0.067 (2 hrs), 0.063 (3 hrs), 0.060 (4 hrs), 0.058 (6 hrs) and 0.052 mg / 100 mg (8 hrs). At the end of treatment period (8 hrs) the cholesterol contents decreased to 26.76%. When glucose was not available cholesterol contents showed a significant (P<0.05) decrease only up to 8 hrs (0.071) for control, 0.068 (30 mins), 0.066 (1 hr), 0.063 (2 hrs), 0.061 (3 hrs), 0.058 (4 hrs), 0.056 (6 hrs), 0.040 (8 hrs). At the end, the decrease was 43.66%. The body cholesterol content was decreased due to starvation which might have been used for the release of energy.

Keywords: *Paramphistomum cervi*, cholesterol, glucose, starvation

Introduction

Cholesterol is a derivative of lipids, which are complex group of bio-molecules to perform different functions in the body of an organism including the amphistome parasites. The main function of these molecules is to perform the framework of the cell membranes and to provide the highest energy yielding molecules. These molecules are mostly reserve fats, which get more energy than carbohydrates and proteins, excess of either proteins or carbohydrates are converted to lipids. Another important function of lipids which will serve an important role in the formation of bile acids in vertebrate host and helps in the absorption of nutrients and acts as co-factors of parasitic enzymes, Symons and Jones (1974). During helminthes infection in the vertebrate host, the lipid content is more, Smyth (1984) and hence acts as immunity to the parasite.

In the present study, the parasites were isolated from the rumen of sheep and they were maintained in an artificially freshly prepared tyrodes solution. Under starvation, the lipids were converted to cholesterol and liberate energy. Therefore, at what levels the lipids are converted to cholesterol for the utilization of energy in the absence of glucose. This helps in drug discovery of antihelmintics to control of helminthes infection in vertebrates. Few and limited literature available in the parasite on cholesterol starvation, such as Bloch (1960), Dawson and Rhoades (1964), Enigk et al. (1970), Padma (1976), Reid (1942), Rittarson (1973), Swamy (1976) and Takagi (1956).

Materials and Methods

For collection of *Paramphistomum cervi* (Zeder, 1790; Fishoeder, 1901) the worms from the rumen of the freshly decapitated sheep from the local slaughter
houses. The rumen was transported to the laboratory in tyrodes solution. In the laboratory, rumens were immediately cut open and the worms were picked up by using a pair of tweezers. After washing the worms thoroughly in normal saline (0.7%) solution, the worms were again washed in an antiseptic containing 1000 units of penicillin/1 ml of the tyrodes solution, to prevent infection to the parasites. Thereafter the worms were maintained in the tyrodes solution for experimental studies. Two experiments were conducted, one for the experimental studies and other for the control. Each experimental studies have five replicates were maintained. Each experiment had 250 worms.

**Treatment 1:** The worms were maintained in tyrodes solution containing glucose (1%) and a control were also maintained only in tyrodes solution along with the same interval of time.

**Treatment 2:** The worms were maintained only in tyrodes solution without glucose. A control in tyrodes solution containing glucose (1%) was also maintained.

**Sampling:** For all treatments, worms were maintained at different time intervals such as 30 mins, 1 hr, 2, 3, 4, 6 and 8 hrs and processed for the estimation of cholesterol. Weigh about 50 mg of the parasite tissue and homogenize in a mechanical homogenizer for 1000 rpm. Then the homogenate was centrifuged in 3000 rpm. Then the supernatant was used for the estimation of cholesterol by the method of Crawford (1958).

**Results and Discussion**

The cholesterol content in the body of *Paramphistomum cervi* and its changes during in vitro isolation with and without glucose are shown in table 1, 2 and fig.1. The data indicates that the cholesterol content in the normal worms is 0.071±0.008 mg/100 mg of tissue. When the glucose was available, the decrease in the cholesterol content from 1.55 percent at the end of 30 mins to 26.75 percent at the end of 8th hrs, Table 1. In the absence of glucose, the decrease in the cholesterol content was rapid ranging from 4.22 percent at the end of 30 mins to 43.66 percent at the end of 8th hrs, Table 2.

The results indicate that the cholesterol content utilized during starvation. Cholesterol synthesizes takes place either by the fats or during from the diet, Smyth (1984) suggested that flat worms do not have the de novo fat synthesizing capacity and they have to be obtained in the diet. When there is no exogenous supply of glucose, the rate of decrease of cholesterol is more and forms into Acetyl-Co. A molecule and then enters into the TCA cycle for the release of energy (Folsch et al., 1957). Further the decrease of cholesterol for the breakdown and release of energy, the lipids are also decreased, as they act as precursors of cholesterol (Kublickione, 1962). Lomukhin (1971) suggested that the parasitic worms do synthesis lipids which in turn synthesis the cholesterol.

Devendra Bansal et al. (2005) suggested that role of cholesterol in parasitic infections internalization of eukaryotic pathogens like Protozoa (Leishmaniasis, Malaria and Toxoplasmosis) and the exchanged of cholesterol along with other metabolites during reproduction in Schistosomes (helminthes) under variable circumstances are poorly understood. In patients infected with some other helminthes alterations in the lipid profile have been observed. Also, the mechanism involved in lipid changes especially in membrane proteins related to parasite infections remains uncertain. Present literature review shows that parasite induce significant changes in lipid parameters, as has been shown in the in vitro study where substitution of serum
by lipid cholesterol in the medium and is experimental model in vivo. These changes in lipid profile occur in patients having active infections with most of the parasites. Membrane proteins are probably involved in such reactions. All parasites may be metabolizing cholesterol, but the exact relationship with pathogenic mechanism is not clear. Some studies suggest that there may be some factors or enzymes which allow parasites to break up and continue lipid cholesterol. Chol et al. (2003) suggested that proteomic changes during disturbance of cholesterol metabolism by azacoprostone treatment in Caenorhabditis elegans by this treatment they can utilize non-functional sterol by converting them into cholesterol and other sterol for cellular functions. This results in serious defect in germ cell development, growth, cuticle development and motility behavior.

**Table 1.** Changes in body cholesterol contents at different time interval (from 30 min-8 hrs) in the presence of glucose (mg/100 mg of tissue)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>30 min</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>0.071</td>
<td>0.07</td>
<td>0.069</td>
<td>0.067</td>
<td>0.063</td>
<td>0.06</td>
<td>0.058</td>
<td>0.052</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.008</td>
<td>±0.008</td>
<td>±0.009</td>
<td>±0.007</td>
<td>±0.009</td>
<td>±0.006</td>
<td>±0.009</td>
<td>±0.008</td>
</tr>
<tr>
<td>Change</td>
<td>-0.001</td>
<td>0.002</td>
<td>0.004</td>
<td>0.008</td>
<td>0.011</td>
<td>0.013</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>-1.55</td>
<td>-3.09</td>
<td>-5.63</td>
<td>-11.26</td>
<td>-15.49</td>
<td>-18.61</td>
<td>-26.76</td>
<td></td>
</tr>
<tr>
<td>P’ value</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Cholesterol contents change at different time intervals of isolation in the absence of glucose (values expressed in mg/100 mg of tissue)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>30 min</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>0.071</td>
<td>0.068</td>
<td>0.066</td>
<td>0.063</td>
<td>0.061</td>
<td>0.058</td>
<td>0.056</td>
<td>0.04</td>
</tr>
<tr>
<td>S.D.</td>
<td>±0.008</td>
<td>±0.004</td>
<td>±0.005</td>
<td>±0.004</td>
<td>±0.005</td>
<td>±0.005</td>
<td>±0.005</td>
<td>±0.006</td>
</tr>
<tr>
<td>Change</td>
<td>-0.003</td>
<td>0.005</td>
<td>0.008</td>
<td>0.01</td>
<td>0.012</td>
<td>0.015</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>% change</td>
<td>-4.22</td>
<td>-7.77</td>
<td>-11.66</td>
<td>-14.75</td>
<td>-18.3</td>
<td>-21.73</td>
<td>-43.66</td>
<td></td>
</tr>
<tr>
<td>P’ value</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Cholesterol content change at different intervals of isolation in the presence and absence of glucose

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