CHAPTER 3

3.3: EXPRESSION OF GLUTATHIONE S-TRANSFERASES IN HUMAN FETAL LIVER.

3.3.0 INTRODUCTION

The major aim of the study was to analyze the expression of GSTs in the normal proliferating cells so a comparison can be drawn with those of cancerous tissues. In this connection human fetal livers from different gestation periods viz; 21, 29, 31 and 33 weeks were studied for the GSTs isoform profile. Further the affinity purified human fetal GSTs were used for substrate specificities and RP-HPLC analysis followed by immunoblotting.

3.3.1 RESULTS

3.3.1.1 AFFINITY PURIFICATION

At different stages of gestation (21, 29, 31, and 33 weeks) human fetal liver tissues were collected from Deccan Medical college, and GSTs were purified by GSH affinity chromatography and the data on purification profile is presented in Table 15. The GSTs activity levels (Fig. 24) showed a change in the 31 week fetal liver which is not in consistence with the protein value as seen in the other gestation week. The purified proteins from fetal livers, on SDS PAGE majority resolved into two bands with 25.0 kDa, 25.6 kDa molecular weights (Fig.23). However in 29 and 31 week
Table 15: Purification Profile of human fetal Hepatic GSTs

<table>
<thead>
<tr>
<th>Gestation week</th>
<th>Total Activity (Units)</th>
<th>Total Protein (mg)</th>
<th>Specific Activity (Units/mg.min protein)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>280</td>
<td>8.1</td>
<td>34.5</td>
<td>45.8</td>
</tr>
<tr>
<td>29</td>
<td>430</td>
<td>8.6</td>
<td>50.0</td>
<td>45.2</td>
</tr>
<tr>
<td>31</td>
<td>620</td>
<td>8.3</td>
<td>74.6</td>
<td>45.8</td>
</tr>
<tr>
<td>33</td>
<td>640</td>
<td>13.4</td>
<td>47.7</td>
<td>46.2</td>
</tr>
</tbody>
</table>

One Unit is defined as one n mole of Thioether formed per min.
Fig. 23: SDS PAGE analysis of affinity purified human fetal liver GSTs

Lane 1: 21 Week Fetal Liver (10 ug)
Lane 2: 29 Week Fetal Liver (10 μg)
Lane 3: 31 Week Fetal Liver (10 μg)
**Lane 4: LMW Markers**
Lane 5: 33 Week Fetal Liver (10 ug)
Fig. 24: Activity levels of liver GSTs from human fetuses at different weeks of gestation

μmol/min/mg of cytosolic protein

Gestation week
fetal livers another additional band with 28.0 kDa MW band was also present.

3.3.1.2 RP-HPLC ANALYSIS

RP-HPLC analysis of the affinity purified GSTs from 21 and 33 week fetal livers showed a single peak with retention times 38.7 min and 38.5 min respectively (Fig. 28 a & d). In case of GSTs from 29 and 31 week fetal livers however, two peaks with retention times around 38 min and 45 min were observed (Fig. 28 b & c). The extra peak observed in the 29 and 31 week fetal liver GSTs (RT~ 45 min) belonged to the additional 28 kDa protein band as observed on SDS-PAGE (Fig 25). To understand the subunit composition of the peak with RT 45.3 min from 29 week fetal liver, two-dimensional gel electrophoresis was performed (Fig 26) from the 2D-PAGE data, the isoelectric point of the protein was found to be around 7.5 to 8.0 pH.

3.3.1.4 SUBSTRATE SPECIFICITIES

Apart from the subunit composition, the substrate specificities of the affinity purified GSTs were also studied. As shown in the table (Table 16), the affinity of the GSTs towards CDNB, ethacrynic acid and 1,2-epoxy-3-propane increased with the age of the fetus, the increase ranging from 2 fold in case of CDNB to 10 fold in case of 1,2-epoxy-3-propane. In case of sulphobromophthalein, the activity was not detected by the GSTs from 21 and 33 week old fetuses. This activity was maximum in the GSTs from 29
Fig. 28: RP-HPLC analysis of GSTs from human fetal livers at different gestational age

Column: Waters μ Bondapak C\textsubscript{18} (0.39 X 30 cm)
Solvent: 0.1% TFA in 20% Acetonitrile (Solvent A)
0.1% TFA in 65% Acetonitrile (Solvent B)
Gradient: Step
Flow rate: \textbf{1 mL/min}
Detection: 214 nm
Sample: 75 μg of affinity GSTs
Fig. 25: SDS PAGE analysis of 38.0 min & 45.8 min RP-HPLC peaks from human fetal liver

Lane 1: RP - HPLC Peak with RT 38.0
Lane 2: Low Molecular Weight Markers
Lane 3: RP - HPLC Peak with RT 45.8
Fig. 26: 2D electrophoresis of 35.4 min RT peak from 29 week old fetal liver (20μg)
Table 16: Substrate Specificities of Human Fetal Hepatic GSTs at different weeks of gestation

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Age of the fetus in weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>21 Week</td>
</tr>
<tr>
<td><strong>1,-chloro 2,4,-dinitrobenzene</strong></td>
<td>280</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>150</td>
</tr>
<tr>
<td><strong>1,2,-Epoxy-3-(p-nitrophenoxy )-propane</strong></td>
<td>20</td>
</tr>
<tr>
<td>Sulfobromophthalein</td>
<td>Nd</td>
</tr>
<tr>
<td><strong>4-Nitropyridine-N-oxide</strong></td>
<td>90</td>
</tr>
<tr>
<td><strong>3,4,-Dichloronitrobenzene</strong></td>
<td>220</td>
</tr>
</tbody>
</table>

*Units of activity: n mole/min.mg protein, nd = not detected*
week fetus followed by that of 31 week old fetus. This transient appearance of activity with sulphobromophthalein was increased in the 29 and 31 week fetal livers, may be due to the extra isoform of GST that was observed only in these two stages.

3.3.1.5 WESTERN BLOT ANALYSIS

In order to characterize the GST subunits expressed during the course of development, GSTs separated on SDS PAGE were transferred to nitrocellulose paper and probed with human α, µ and π class specific GST antibodies (Fig 27). As shown in the Fig.27, α form of GSTs were present in the livers from all the stages of gestation periods studied. But in the 29 and 31 week fetal liver apart from the two bands another band was also recognized by the α class specific antibodies, similar to that seen on SDS PAGE. However no protein was detected when the GSTs separated on SDS PAGE were probed with µ and n class specific antibodies.

3.3.1.6 DISCUSSION

GSTs as the detoxifying enzymes play an important protective role in fetal demise. Though many reports have been published regarding the GSTs profiles in developing tissues none of the studies were complete as the availability of fetal tissue is a scarcity. Greengard (Greengard et al; 1970) proposed that changes in the expression of liver-specific proteins generally occurred at three specific developmental stages, (I) Late gestation, (II) at or directly after birth and (III) just before weanling. During
Fig. 27: Immunoblot analysis of fetal liver GSTs, probed with a class specific antibodies.

Affinity Purified GSTs from livers of 33 week (lane 1), 31 week (lane 2), 29 week (lane 3) and 21 week (lane 4) human fetuses. 10 μg of protein was loaded in each lane.
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these periods the liver undergoes a significant anatomical, morphological and physiological changes in response to (a) commitment of embryonal cells to become hepatocytes, (b) preparation for extra-uterine function during late gestation and (c) maturation of newborn liver.

In the present study we observed that there is a variation in the specific activity during the course of gestation, suggesting that there could be an ontogenic change in the titer of different isoforms of GSTs, which was also observed by K.Datta (Kaushik Datta et.al 1994). The substrate specificity studies have further reveled that there will be a variation in the GSTs profiles Gutenberg (Gutenberg et.al; 1986) reported two distinct forms of GSTs in human fetal livers an alpha and a pi form. But in the present study only one form of GST was found is alpha and no pi or mu forms were detected. This observation is in consonance with with the report published by Sato (Sato.K et.al; 1989). This could be because of the difference in gestation weeks, Gutenberg studied in the early term fetal livers where as the present study was on late term fetal livers. Strange (Strange R C. et.al; 1985) while studying GSTs from fetal tissues observed three subunits of GSTs and termed them as GST1, GST2 and GST3. They also have reported that, after 30 weeks of gestation the GST3 decreases, which is in accordance with the present findings. Edward (Edward et.al; 1977) had proposed that human development is often accompanied by a generalized increase in the expression of basic
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Isoenzymes and decreased expression of acidic forms of GSTs in fetal livers. A continuous expression of GST alpha in fetal livers with minor changes was reported during the course of gestation (Strange et al., 1984). On the contrary the pi form of GST was reported only in the early stages of development (Van lieshout et al., 1998). In all these studies no effort is made to study the changes in individual subunits and the stages at which variations take place.

Increase in the Ya form of GST during the course of development especially in the late term of pregnancy was reported in rats (Listowsky et al.,) which is in accordance with the present study. Apart from this they have observed the presence of Yp (p form) which decreased as age progressed. In the present study, however no p form of GST was observed, may be because the studies were concentrated on the late term fetuses.

In case of rat development, a differential expression of GSTs was observed with reference to the two subunits of GST alpha (Lisa B.G.Tee et.al 1992), Subunit 1 was found to be dominant in adult liver where as subunit 2 fetal liver. This clearly shows that there is a developmental regulation of GST in mammalian systems.

The physiological significance for the presence of alpha form of GSTs in the liver is not quite clear. However, it can be proposed that the a form of GSTs with high peroxiciases activity might be playing a role in the
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protection of the fetus during pregnancy from the oxidative stress. The extra uterine environment is markedly hyperoxic compared to that of fetus (Bruke et.al 1978). This could be the reason for the increase in the alpha forms of GSTs in the late term of human fetal liver, which is also seen in rat liver development (Lisa et.al 1992). Though different GST isoforms share common substrates such as CDNB, the varied developmental expression emphasizes that their invivo substrates are likely to be very different from those of invitro

From the foregoing studies it is quite clear that in actively proliferating normal fetal tissues a form of GSTs are predominant. This observation, however, is quite different from those of actively proliferating abnormal tissues such as cancer, where in p is the predominant form of GST. In these tissues, the a form is on the other hand is decreased. This differential expression of GST isoforms in actively proliferating normal and pathological tissues, may be due to the varied environments to which the tissues are exposed. Further studies, however, are required to understand the role of a form of GSTs in normal dividing tissues and n form of GSTs in cancer tissues.