Reduction of liver folates in gossypol-treated rats

Gossypol is a yellowish phenolic compound occurring naturally in certain species of cotton plants of the family Malvaceae, mostly in the seeds and root bark. At one time it was considered only as a toxic waste in the processing of cotton seed products. However, following the initial observation of Liu in China in 1957, the pioneering work of other Chinese workers in the mid-eighties and confirmation by several others established its role as a male antifertility agent both in laboratory animals and humans. There was a spurt of papers in the late eighties and early nineties on the antifertility effect of gossypol and WHO task force on male contraceptives even conducted extensive clinical trials on gossypol in China and elsewhere.

Though in several studies, its efficacy was well-proven with apparently no side-effects, in a couple of other studies toxic symptoms like anorexia, reduction in body weight, hypokalemia, etc., were observed even at low doses. It was reported by Menaul as early as 1923 that gossypol prevents liberation of oxygen from oxyhaemoglobin and has haemolytic effect on erythrocytes. In fact, studies by Danke and Tilman showed that microcytic hypochromic anaemia occurs in rats when 10% cotton seed meal is fed for a period of 28 days. Since folic acid is involved in erythropoiesis and its deficiency leads to anaemia, we wanted to study the effect of gossypol at antifertility doses on liver folate levels in rats. Graded doses of gossypol acetic acid (Sigma Co. 20, 30 mg/kg body weight/day) were given through gavage for different durations (5, 7 weeks) to male rats of Wistar/Nin strain. The animals were fed stock colony diet with adequate levels of folic acid (1 mg/kg diet). At the end of treatment and after mating the animals with virgin females to assess the fertility, the animals were sacrificed. Liver was excised and weighed and a portion of the liver was processed to determine the folate levels as per the method of Lakshmaiah and Ramasastri.

The rats became totally infertile at the dose regimen of 30 mg/kg body weight/day for 7 weeks, as reported earlier. Liver folate levels in gossypol-treated animals have not been reported so far and, to the best of our knowledge, this is the first study of the effect of gossypol on folic acid in vivo. The data are presented in Table 1. Liver weights were normal in all the treated groups. There was no difference in liver folate levels of treated rats at 20 mg regimen fed for 7 weeks. But at higher doses, i.e. 30 mg regimen, for 7 weeks there was significant reduction from control ($P < 0.001$) and this was seen as early as 5 weeks ($P < 0.05$). The dose effect was very evident, on comparing the 20 mg regimen with 30 mg regimen for 7 weeks ($P < 0.05$). Tissue distribution studies show that liver accumulates large amounts of gossypol and because of its slow clearance from the body it is quite possible that over a prolonged period of ingestion, toxic effects may appear even at low antifertility doses. The reduction in liver folate levels in gossypol-treated animals may be due to the direct action of gossypol on liver, as some earlier studies have shown hepatotoxic effect of gossypol. However, no apparent toxic symptoms were visible in these animals throughout the experimental period as the food intake was normal, and body weights were not affected.

Table 1. Changes in hepatic folic acid in gossypol-treated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of exp (weeks)</th>
<th>Gossypol acetic acid dosage (mg/kg body w/day)</th>
<th>Liver wt (mean ± SE) (L. casey activity)</th>
<th>Liver folate µg/g liver (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>10 ± 0.16 (4)</td>
<td>4 ± 0.01 (4)</td>
</tr>
<tr>
<td>Gossypol-treated</td>
<td>5</td>
<td>30</td>
<td>10 ± 0.42 (6)</td>
<td>4 ± 0.13*</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>-</td>
<td>10 ± 0.82 (6)</td>
<td>4 ± 0.23*</td>
</tr>
<tr>
<td>Gossypol-treated</td>
<td>7</td>
<td>20</td>
<td>10 ± 0.33 (5)</td>
<td>3 ± 0.10*</td>
</tr>
<tr>
<td>Gossypol-treated</td>
<td>7</td>
<td>30</td>
<td>10 ± 0.30 (6)</td>
<td>2 ± 0.24*</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate number of animals

$*P < 0.05$ by modified $t$ test, compared to control, 5 weeks. Values not sharing common superscript were significantly different by analysis of variance, and stuffed range test. $^{* * P < 0.001}$, $^{* P < 0.05}$.
HBV: Have we found the ultimate answer?

S. P. Thyagarajan

Hepatitis B virus (HBV) continues to be the single most important cause of viral hepatitis throughout the world, along with hepatitis C virus, an important cause of chronic liver diseases and hepatocellular carcinoma. The situation is further disturbing as seen from the recent reports that HBV could also be involved in extra-hepatic immunologically mediated diseases like primary biliary cirrhosis, polyarteritis nodosa and glomerulonephritis.

The only known reservoir of this notorious virus is human beings themselves as healthy carriers of HBV in all the populations in the world. To date, there are nearly 370 million HBV carriers in the world, with the highest incidence of 10–20% in the tropical countries. In India itself, a conservative estimate of 30 million carriers is projected to be present based on 3–5% HBV carrier rate in the population.

Studies on HBV exposure pattern in the population have clearly shown the predominant role of horizontal transmission besides vertical/perinatal transmission in the spread of HBV. Reports have also shown the family clustering of HBsAg in 40–65% of family members of HBV carrier families, while the same was much lower in non-carrier families.

Prevention and control of hepatitis B

All these informations on the notority of HBV led to public health urgency of an effective prevention strategy for hepatitis B. As per the conventional methodology adopted in the prevention of any infectious disease, production of hepatitis B vaccines was successfully attempted in the 1970s itself.

Plasma-derived hepatitis B vaccines

The first of the hepatitis B vaccines was manufactured using hepatitis B surface antigen (HBsAg) particles from the plasma of chronic HBV carriers by Krugman and Giles in 1973 (ref. 7). However, the commercial plasma-derived hepatitis B vaccine came into human use only in 1982. Current plasma-derived vaccines consist of highly purified formalin-inactivated and/or heat-inactivated alum-adsorbed hepatitis B 22 nm subviral particles of HBsAg that are free of detectable nucleic acid. The antigen is harvested from the plasma of asymptomatic, apparently healthy human carriers of HBV by a series of steps that may include precipitation, ultracentrifugation, gel filtration and/or affinity chromatography.

The methodology of this vaccine preparation is in brief as follows:

- Plasma from hepatitis B carriers
- Defibrination (with added calcium)
- Ammonium sulphate precipitation (concentration)
- Isoyptic banding (sodium bromide)
- Rate zonal sedimentation (sucrose gradient)

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COMMENTARY

Pepsin digestion pH2 (10-fold purification)
- Urea 8 M (denature-renature)
- Gel filtration (molecular sieve)
- Formalin 1:4000 (72 h/37°C)
- Vaccine, 20 mcg surface antigen/dose with 0.5 mg alum in 1 ml adjuvants.

By these stringent procedures it is stated that all the known blood-borne viruses like retro, including HIV 1 and II, toga, lenti (HCV) and others are inactivated fully and their nucleic acid components removed.

Currently, there are more than a dozen manufacturers of plasma-derived vaccines worldwide. The commonest ones are Hepatavax B, manufactured by Merck, Sharp and Dohme laboratories, USA, and Hepavac-B by Korean Green Cross Corporation. These vaccines have been proved by several clinical trials as safe, immunogenic and effective hepatitis B vaccines.

Genetic recombinant hepatitis B vaccines

The widespread use of plasma-derived vaccines was curtailed by relatively expensive production costs and unfounded fear that resistant or unknown organisms could escape inactivation during the preparation of the plasma-derived vaccine. Elaborate procedures are necessary to purify the vaccine, and time-consuming tests must be performed to ensure that the vaccine is free from