In vivo model for dyslipidemia with diabetes mellitus in hamster*

Gitika Bhatia**, Farhan Rizvi, Rashmi Saxena, Anju Puri, A K Khanna, Ramesh Chander†, E M Wulff* & A K Rastogi

Division of Biochemistry & **Toxicology, Central Drug Research Institute, Lucknow, 226001, India
†Division of Health Care Discovery and Pre-clinical Development, Novo Nordisk, Denmark

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Due to similarities in lipid metabolism to those in humans, hamster is considered as a good model for the study of regulatory mechanisms of plasma lipoproteins in response to cholesterol or fatty acid-enriched diet. This model of hyperlipidemia has been modified to produce dyslipidemia with diabetes complexities by feeding with high fat diet added with 9% (w/w) fructose. Feeding this diet to hamster for 10 days markedly increases plasma levels of triglyceride, cholesterol, fatty acids followed by a significant increase in glycerol, β lipoproteins, high density lipoprotein, glucose and glycosylated proteins. This model is being used for research and development of lipid lowering drugs with hypoglycemic activity in collaboration with Novo Nordisk, Denmark. The modified high fat diet formulation has now been prepared (Research diet D.99122211) and supplied by Research Diets Inc, Burnswick USA.

Keywords: Diabetes, Dyslipidemia, Hyperglycemia, Lipid lowering drugs, In vivo model

Diabetes mellitus is an independent risk factor for the development of coronary artery diseases, myocardial infarction, hypertension and dyslipidemia. Clinically, diabetic patients are characterized by marked increase in blood glucose level followed by normal or mild hyperlipidemia. Experimental animal models in which diabetes is induced by administration of alloxan, streptozotocin or other agents have been used effectively to study etiologies, complications, treatment and prevention of disease. Many plants like Allium cepa, Allium sativum, Ficus bengalensis, Gymnema sylvestre, Pterocarpus marsupium, Trigintella foenum-graecum, Engenia jambolana etc. have been shown to possess potent anti diabetic activity. According to Shukla et al., some compounds have been purified from T. foenum-graecum, E. jambolana and F. bengalensis, which have both hypoglycemic and hypolipidemic (including hypocholesterolemic) activity and patents have been applied in India and USA. On the other hand chronic disorders of lipid metabolism, dyslipidemia and cardiovascular diseases are together increasing the risk of diabetes complexes in patients. Such disorders are primarily characterized by dyslipidemia, (predominantly hyperlipidemia type IV), glucose intolerance with mild increase in blood glucose. With the aim to control these ailments a drug having 2-fold properties i.e. lowering of the blood lipids and glucose together is in great demand. In research and development of antidiyslipidemic drugs the animal models are extensively created through feeding with cholesterol rich high fat diet (HFD). These animals, after a long treatment with HFD produced hyperlipidemia with mild hyperglycemia. For rapid screening of antidiyslipidemic drugs the selection of an appropriate animal model that closely resembles with humans for the study of dyslipidemia-diabetic complexities and development of antidiyslipidemic drugs must be done with considerable thought and compromise. The hamster, guinea pigs and rabbit easily accumulated large amount of serum and liver lipids when fed with diet supplemented with cholesterol and cholic acid. Lipid lowering activity of many drugs including garlic was evaluated in HFD fed hyperlipidemic rabbits. Further, feeding with high fructose to these animals frequently develops conditions of insulin resistance. Fructose rich diet is also known to enhance hepatic secretions of very low-density lipoprotein (VLDL), thus resulting in hyper triglyceridiemia, but not dyslipidemia in animals. The level of plasma lipids, glucose and lipoprotein metabolism in syrian hamster are almost similar to those in humans but there are certain limitations, hamsters do not develop atherosclerosis even though they rapidly develop hyperlipidemia. Hamster does not resemble humans due to...
genetic factor and cannot be used as an experimental model for diabetes. For this only dd db mice are accepted.

With an aim to choose hamster as a model of dyslipidemia with diabetes complexities, an appropriate amount of fructose (9% w/w) was added in high fat diet containing cholesterol (0.45% w/w) in coconut oil and deoxycholic acid (0.45% w/w). Feeding with this diet for 10 days rapidly induced severe dyslipidemia followed by significant increase in plasma glucose and glycosylated proteins. The anti-dyslipidemic effect of fenofibrate has also been confirmed in this model.

Cholesterol, deoxycholic acid, fenofibrate were purchased from Sigma Chemical Co., USA. Refined coconut oil was purchased locally and washed with 10% Na₂HCO₃ to obtain the acid value up to 2.5 of the oil.

Composition of high fat diet—Fructose (100 g), deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered hamster chow diet. Simultaneously cholesterol (5 g) was dissolved in 300 ml warm coconut oil supplied by Ashirvad Industries, Chandigarh, India. This oil solution of cholesterol (5 g) was added slowly into powdered mixture to obtain homogenous soft cake. This was moulded in the shape of pellets of about 3 g each. This high fat diet (HFD) is now prepared by Research Diet Inc., New Brunswick, USA (product Code No. D99122211) and supplied through Novo Nordisk.

Preparation of drug—Fenofibrate (mol wt 360.8) was suspended (100 μmol/ml) in standard vehicle containing 0.2% carboxymethyl cellulose + 0.4% tween 80 in distilled water.

Animals—Male Golden Syrian hamsters, weighing 120-130 g, bred in the animal house of the Institute were used. These animals were divided into 3 groups control, dyslipidemic and dyslipidemic plus drug treated groups of 8 each and kept in a room controlled for temperature (25°C ± 2.0°C) and 12/12 hr L: D cycle (light on at 08:00 hrs). The animals had free access to the HFD and water for 10 days (Day 1 to day 10).

Fenofibrate suspension was fed orally at a dose of 1000 μmol/kg body weights once daily from day 4 to day 10. At the end of the experiment body weight and food consumed by animals were recorded. Hamsters (non fasted) were anaesthetized and blood (1 ml) was withdrawn from retro orbital plexus in eppendorf tubes coated with EDTA. The same amount of blood was also taken in eppendorf tubes containing 120 μl sodium fluoride (45mg/ml). The tubes were centrifuged and plasma was aspirated out. The plasma samples without NaF were used for the assay of total cholesterol (TC)17, triglyceride (TG)15 and high-density lipoprotein (HDL)15. However those added with NaF were used for the assay of glycerol (Gly)14, glucose (Glu)15 and free fatty acid (FFA)16 by standard enzymatic methods on auto-analyzer of Beckman Coulter model “Synchron-CX-5 Clinical System”. All assay kits except that of FFA were purchased from Beckman Coulter International USA. Assay Kit for FFA was purchased from Wako Pure Chemical Industries Ltd., Osaka Japan. Plasma without NaF was also used to estimate glycosylated protein according to the method of Goldstein et al17. Very low and low-density lipoprotein (VLDL and LDL) cholesterol were calculated according to Fried Wald formula18:

\[
\text{VLDL-Chol} = \frac{Tg}{5}
\]

All the values are in mg/dl plasma.

Table 1—Effect of fenofibrate (Feno) on physical and plasma biochemical parameters in dyslipidemic hamsters.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wtb (g)</th>
<th>Diet intakec</th>
<th>Tgb</th>
<th>T-Cholb</th>
<th>HDL-Cholb</th>
<th>LDL-Cholb</th>
<th>VLDL-Cholb</th>
<th>FFAb</th>
<th>Glycosylated protein d</th>
<th>Glucoseb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130±5</td>
<td>10.5±0.61</td>
<td>3.44±0.16</td>
<td>1.35±0.12</td>
<td>1.70±0.34</td>
<td>0.384±0.15</td>
<td>151±0.20</td>
<td>0.00±0.34</td>
<td>39.9±4.97</td>
<td>8.00±1.65</td>
</tr>
<tr>
<td>HFD</td>
<td>137.3±5.03</td>
<td>7.5±0.75</td>
<td>17.24±0.23</td>
<td>10.81±0.16</td>
<td>5.75±0.45</td>
<td>3.45±0.27</td>
<td>476±1.27</td>
<td>2.67±0.65</td>
<td>112.7±12.8</td>
<td>57.76±6.57</td>
</tr>
<tr>
<td>HFD+Feno (1000 μmol/kg)</td>
<td>135.5±4.78</td>
<td>6.78±0.41</td>
<td>12.4±0.23</td>
<td>7.64±0.16</td>
<td>4.57±0.82</td>
<td>0.324±0.12</td>
<td>224±0.27</td>
<td>1.27±0.47</td>
<td>57.76±7.02</td>
<td>57.76±7.02</td>
</tr>
</tbody>
</table>

Units:
b: g; cm³; μM; μ mol; g mol fructose/mg protein
p values: *<0.05; **<0.01; ***<0.001; NS not significant

NOTES

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Feeding with HFD in hamsters markedly increases their plasma levels of Tg, TC, β-lipoproteins (VLDL and LDL cholesterol, glycerol and FFA by 9, 3, 9, 3, and 3 fold along with increase in the levels of glucose and glycosylated proteins by 2 and 2 fold respectively as compared to control (Table 1). Administration of fenofibrate (1000 μmol/kg) simultaneously with HFD caused significant lowering in the plasma levels of Tg, TC, VLDL-chol, LDL-chol, glycerol, FFA, glucose and glycosylated proteins by 76, 23, 76, 10, 52, 53, 33 and 21% respectively in drug treated group. HFD caused increase in the plasma level of HDL-chol, which was further increased (33%) after treatment with fenofibrate. It was observed that hamsters like to eat normal pellet diet as compared to HFD. Fenofibrate feeding caused slight and non-significant decrease in the consumption of HFD. However no significant change was found in the body weight of animals among all the three groups.

In the present study, by feeding with fructose rich HFD in hamster model a relationship between dyslipidemia and hyperglycemia was established. It has been reported that hamster has higher lipid demand for its growth in comparison to other members of its family, Cricetinae19. This hamster model is a reversible model; when HFD is withdrawn, the lipid level becomes normal. The desirable hyperlipidemia, which is sufficient to screen antidysslipidemic compound/drugs, is attained in short duration and without any lipotoxicity. However if HFD is prolonged for chronic development one comes across the harmful effects of high load of lipid on hamster. The level of a chronic hypertriglyceridemia above 9 mM (800 mg/dl) is enough to produce high fat load causing acute pancreatitis and to disrupt the membrane integrity due to alterations in lipid composition of muscle and liver20. These changes may desensitize the insulin receptors, fail to recognize glucose transporter proteins and reduce the glucose uptake in muscles and liver. Simultaneously HFD induced pancreatitis may also impair the insulin secretion. Therefore this model is suitable for research and development in dyslipidemia-hyperglycemia complexities and insulin resistance. Alloxan is used to produce selective destruction of the β-cells in islet of Langerhans 21 and induce diabetes with mild hypertriglyceridemia in experimental animals. These models mechanistically are totally different to those of diet-induced dyslipidemia with hyperglycemia and are suitable for screening the drugs for regeneration of damaged pancreatic β-cells in diabetes22. The drug fenofibrate is used as a positive standard drug to show that the model works. Reversal of dyslipidemia simultaneously regulated the level of glucose in dyslipidemic hamster treated with fenofibrate. In view of the above it was observed that hamster is sensitive to diet for induction of dyslipidemia with hyperglycemia and the model may be more appropriate for development of antidysslipidemic drugs with PPAR-α agonistic property23. However the limitations mentioned above have to keep in view. Using this model, more than 300 samples were screened and few among them are in mechanistic evaluation for their antidysslipidemic - hyperglycemic activities.

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