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Research Paper

A Preliminary study on the reproductive toxicity of statins in rats

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Abstract

Statins are reported to have a negative impact on steroidogenesis affecting reproduction, especially pregnancy. The aim of the present study was to evaluate the adverse effects of statins on the maintenance of pregnancy in Sprague-Dawley rats. Sprague-Dawley rats were given vehicle, pravastatin, atorvastatin or simvastatin by oral gavage at dose levels 5, 10 and 20 mg/kg/day and simvastatin (20 mg/kg/day) alone, in supplementation with hydroxy progesterone caproate, 5 mg/day or mevalonic acid lactone, 100 mg/kg/day during pregnancy (2-18 days) and were sacrificed on day 20. Pre- and post implantation loss was noted by performing laparotomy. Hormone analysis and histological evaluation of the uterus was performed in another group of rats receiving the drug or vehicle upto day 11 and sacrificed on day 12. As compared to pravastatin and atorvastatin, simvastatin induced significant implantation loss at 20 mg/kg/day dose level. Co-administration of a progestin could not stop fetal wastage but mevalonic acid supplementation could reverse the effect of simvastatin. The results substantiate that simvastatin emerged as the most active statin towards inducing fetotoxicity and exhibited total interceptive effect leading to loss of all implantation sites producing no live or dead embryos. The process of interception may be through changes in the implantation site and altered hormone level.

Keywords: fetotoxicity, implantation, pregnancy, statin , steroidogenesis

Introduction

Obesity is an important worldwide public health problem. One of the most deleterious metabolic derangements of the disease is hyperlipidemia. In western countries, coronary artery diseases (CAD) related to the primary risk factor of hypercholesterolemia represent the most vital causes of death [1]. In subjects with a regular lipid metabolism, only one third of the total body cholesterol is diet derived and two third being synthesized directly from intracellular precursors by various organs of the body [2]. For these reasons, the control of cholesterogenesis by inhibiting its biosynthesis is an important means of lowering plasma cholesterol levels.

Statins are a class of molecules that inhibit the microsomal 3-hydroxy-3 methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the biosynthesis of cholesterol in the liver and other tissues [3] and so they are widely used clinically for the treatment of hyperlipidemia and for reducing morbidity and mortality in CAD [4,5]. Due to their cholesterol lowering properties, this class of inhibitors might be expected to have adverse effects on reproduction by reducing the supply of circulating

cholesterol, which is required for steroidogenesis [6].

Steroid hormones derived from cholesterol, as well as mevalonate and its isoprenoid derivatives provide important contributions to the maternal animal during pregnancy and lactation [7,8]. The enzyme HMG-CoA reductase catalyzes the rate-limiting step in the cholesterol biosynthesis pathway, so inhibition of this enzyme by agents such as statins result in reduced levels of mevalonate and subsequent products, thus affecting conception.

Reports on animal studies conducted with statins are few. Simvastatin reduced fetal and offspring body weights when given to female rats prior to mating and during organogenesis [9,10]. No adverse effects on reproduction, fertility or survival of the conceptus occurred to male or female rats following oral administration of atorvastatin [11]. However, oral administration of maternally toxic doses of atorvastatin to pregnant rats and rabbits resulted in developmental toxicity, which ultimately led to increased post-implantation loss and decreased fetal body weight, but not teratogenecity [12].

Despite the claim of various effects of statins, there is no published scientific evidence that has substantiated the reproductive toxicity of statins especially simvastatin on rats. In this study, evidence to the fact that the adverse effects of maternal exposure to simvastatin on the initiation and maintenance of pregnancy has been provided in an attempt to get a picture of the reproductive toxicity of the drug. These results have been compared with those of pravastatin and atorvastatin.

Materials and Methods

Animals: Adult Sprague-Dawley rats (180-220 g) were maintained under good husbandry conditions supported by diurnal cycles of 12h light and 12h darkness. They were given access to standard pelleted food (carbohydrate, 65.5%, protein, 21.0%, fat, 5.5%, mineral mixture, 7.0% and vitamin mixture, 1.0%) and filtered tap water *ad libitum* and were acquired from our Institute's animal facility. The animals were acclimatized to the laboratory environment one week prior to the experiments. Adult female rats showing regular estrous cycles were mated with proven fertile males of the same strain, and the presence of sperm in the vaginal lavage was assigned as Day 1 of pregnancy. Mated females were distributed on a random basis into fourteen groups (Gr.1-Gr.14) of 8 rats each or more and were housed individually. The animals were administered the formulated drug or the vehicle orally, using a metal feeding needle. In each group, laparotomy was performed on each animal on day 8 under light ether anaesthesia and the abdominal incision was closed. The animals were followed-up till day 11 or day 19 of pregnancy. The animals were sacrificed either on day 12 or on day 20 of pregnancy. All experiments were performed in accordance with the guidelines formulated by the animal ethics committee of the Indian Institute of Chemical Biology, Kolkata.

Drugs and Dosing: The rats were dosed once daily with pravastatin (Pravator, Solus [Ranbaxy Laboratories Ltd.], India), atorvastatin (Aztor, Sun Pharmaceutical Industries Ltd., India) and simvastatin (Simvotin, Ranbaxy Pharmaceuticals Inc., India) at a dose of 5 mg/kg/day, 10 mg/kg/day and 20 mg/kg/day from day 2-18 of pregnancy (Gr.2-Gr.10, n=8). Dosing suspensions of each statin were freshly prepared in 1 ml distilled water. Vehicle-treated controls received equal volume of distilled water for a similar duration (Gr.1, n=8).

Supplementation of hydroxyprogesterone caproate and mevalonic acid: A group of rats receiving the drug simvastatin at the dose of 20 mg/kg/day were simultaneously supplemented with either proluton depot, hydroxyprogesterone caproate (German Remedies Limited, Mumbai, India), at a dose of 5 mg/day (Gr.11, n=10) or mevalonic acid lactone (Sigma Chemical Co., St. Louis, MO, USA) (Gr.12, n=11) at a dose of 100 mg/kg /day for the period of day 2-18 of gestation and were sacrificed on day 20.
Another group of rats receiving the drug (Gr.13, n=10) or the vehicle (Gr.14, n=10) alone upto day 11 were sacrificed on day 12.

Pre and Post-implantation loss: The study was undertaken on Gr.1 to Gr.10. A laparotomy was performed on each animal on day 8 under light ether anesthesia. Luteal spot and implantation sites were counted and pre- implantation loss was calculated as follows:

No of luteal spots – No of implantation sites

The abdominal incision was closed and the animals were followed up to day 19. The animals were sacrificed on day 20 of pregnancy. Post implantation loss was calculated as follows:
No. of implantation sites − No. of live fetus

Hormone analysis: On the day of sacrifice (gestation day 20) blood samples were collected by direct cardiac puncture (Gr.1, Gr.10, Gr.12) under light ether anaesthesia, serum was separated by centrifugation and stored at -70ºC. Serum progesterone and estradiol were measured using kit materials for double antibody radioimmunoassay (Diagnostic Products Corporation). The detection limit was 0.05 ng/ml and 1.4 pg/ml for progesterone and estrogen respectively.

Histological evaluation: The uterus were removed from day 12 pregnant animals (Gr.13 and Gr.14), fixed in Bouin's solution for 24 h at room temperature. For light microscopy examination, tissues were embedded in paraffin, sectioned at 5µm thickness, stained with hematoxylin-eosin for morphological analysis of the uterine epithelium and photographed with Kodak colour photoautoprint.

Statistical analysis: The difference in the mean number of implantation sites and hormone parameters in the treated versus control groups was analysed by applying Duncan's Multiple Range test (Graphpad Prism software version 5). The values were expressed as mean ± SEM. The significance level was set at $p<0.05$ for all test.

Results

Effect of statins on post-implantation loss: In the present experiment, it was observed that pre-implantation loss was insignificant in all the groups with the three different statins administered (unpublished data). At lower doses (5 & 10 m*g*/kg/day), pravastatin, atorvastatin and simvastatin did not show significant fetal wastage as compared to control. On administration of 20 m*g*/kg/day pravastatin and atorvastatin, appreciable ($p<0.05$) implantation loss was evident (Figures 1 & 2). However, significantly higher incidences of resorptions ($p<0.001$) (Figure 3) were encountered in animals treated with 20 mg/kg/day simvastatin.

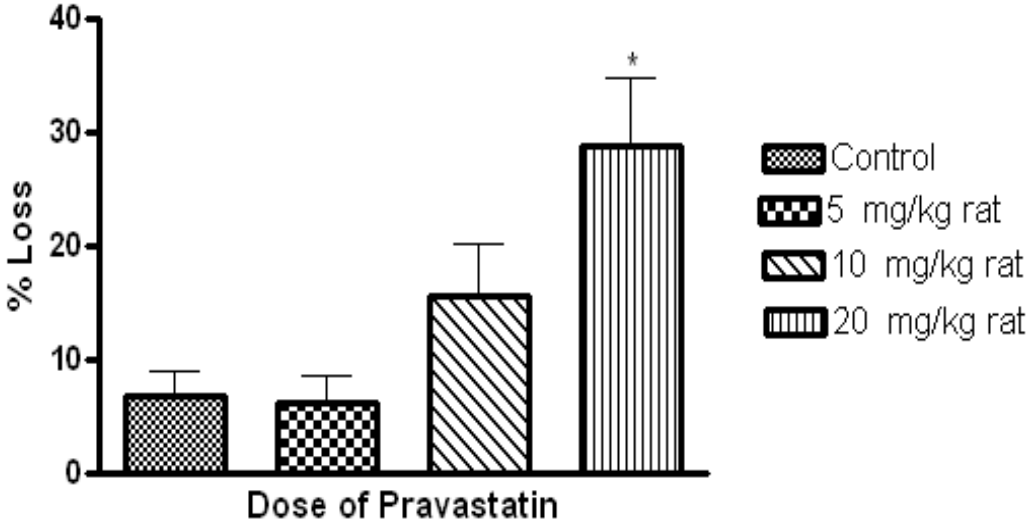


Figure 1: Data represents the effect of different dosages of pravastatin in rats during gestational phase (2-18 days). Results are shown as mean ± SEM. *p<0.05, compared to control group

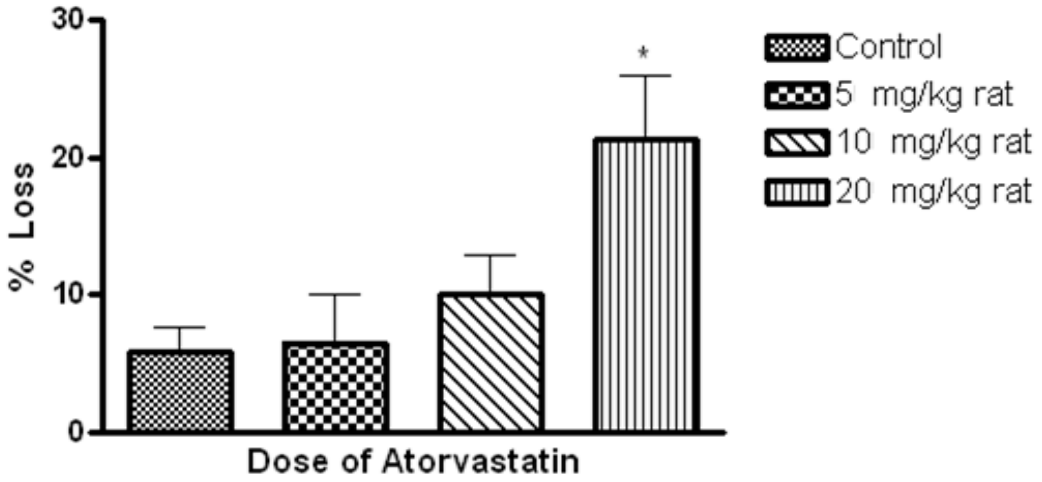


Figure 2: Data represents the effect of different dosages of atorvastatin in rats during gestational phase (2-18 days). Results are shown as mean ± SEM.*p<0.05, compared to control group

Figure 3: Data represents the effect of different dosages of simvastatin in rats during gestational phase (2-18 days). Results are shown as mean ± SEM. ***p<0.001, compared to control group

Effect of hydroxyprogesterone caproate or mevalonic acid on simvastatin-treated rats: Administration of hydroxyprogesterone caproate along with simvastatin could not stop loss of pregnancy. Loss is statistically significant ($p<0.001$). However, the cholesterol synthesis intermediate, mevalonic acid was found to be effective in reversing simvastatin-induced interceptive effect. Simvastatin-induced pregnancy loss was restricted by mevalonic acid which was not statistically significant (Figure 4).

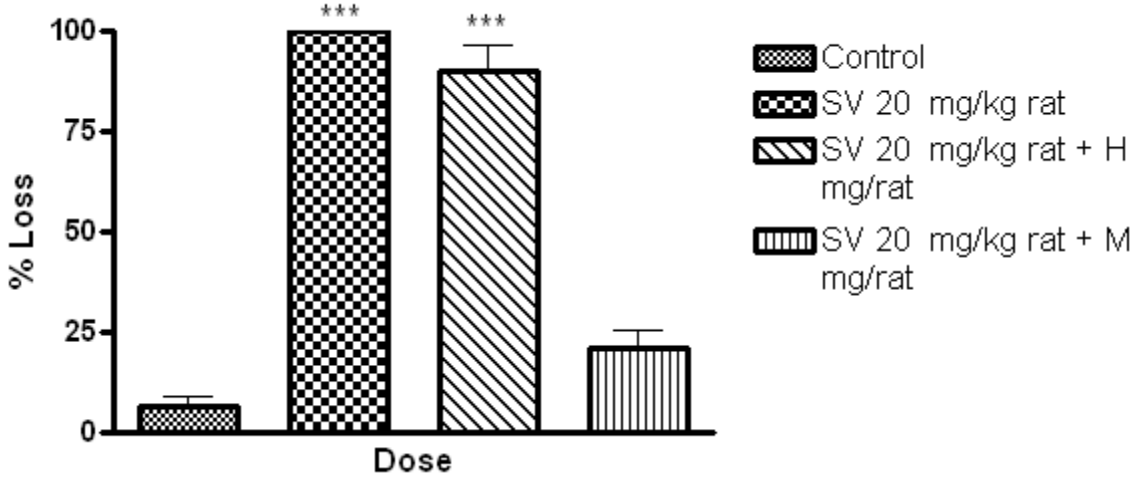


Figure 4: Data represents the effect of Hydroxyprogestrone caproate (H) and Mevalonic acid (M) supplementation on simvastatin (SV)-induced wastage of pregnancy. Results are shown as mean±SEM. ***p<0.001, compared to control group.

Hormone analysis: Gavage of simvastatin (20 mg/kg/day) reduced the serum estradiol level by 41.1% as compared to control. Gavage of simvastatin along with injection of mevalonic acid caused reduction of estrogen level to 20.0% as compared to control (Table 1). In contrast, progesterone level in simvastatin-treated group was reduced to 43.0% as compared to control group. When mevalonic acid was administered along with simvastatin, the progesterone level came down to 70.7% as compared to control. Thus, mevalonic acid administered along with simvastatin contributed in minimising reduction in estrogen and progesterone levels.

Table 1: Effect of simvastatin on plasma steroids in rats

Groups	Estrogen (pg/ml)	Progesterone (ng/ml)
Control	81.6±9.4	106.0±19.8
Simvastatin	33.5±12.2	45.6±21.4
Simvastatin+ mevalonic acid	65.3±6.9	75.0±6.1

Values are expressed as mean ± SEM. Control and treated values differ significantly at $p<0.05$.

Histological evaluation: Histological picture in control rats (Gr.14, gestation day 2-12) (Figure 5) indicated the presence of live fetus whereas simvastatin-treated rats (Gr.13, gestation day 2-12) showed a smooth endometrial luminal epithelium but no sign of fetus (Figures 6a and 6b). The stroma was compact and multiple numbers of endometrial glands were present as visible in both the figures. Thus, administration of simvastatin to gestation day 2-12 treated rats induced fetal resorption and showed prominent changes of wide endometrial lumen with glands in the stroma.

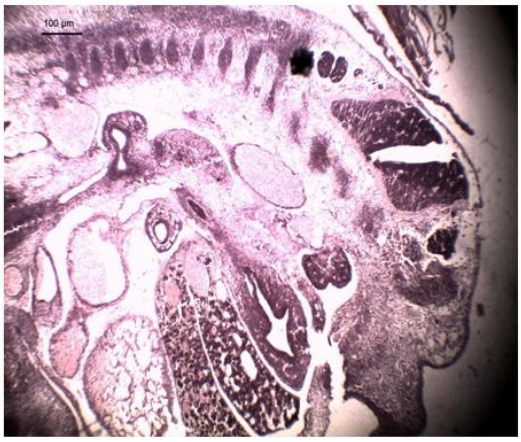
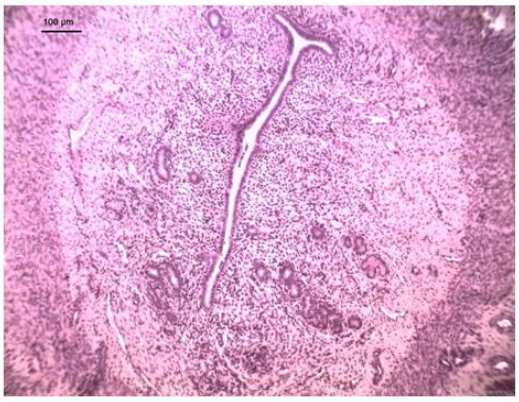
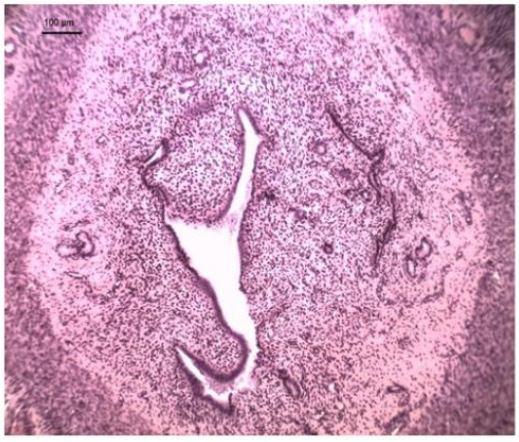


Figure 5: Section of control rat uterus indicating well-preserved viable fetus (Hematoxylin-Eosin, original magnification x 4)



(a)



(b)

Figure 6: (a) Section of rat uterus treated with simvastatin (20 mg/kg/day) indicating fetal resorption and well-preserved endometrial gland. (Hematoxylin-Eosin, original magnification x 4) (b) Section of rat treated with simvastatin (20 mg/kg/day), developed a wide lumen. (Hematoxylin-Eosin, original magnification x 4)

Discussion

The lipid lowering agents known as statins, are known to reduce the intracellular concentration of cholesterol and provide significant protection against coronary artery disease [13]. Reports suggest that inhibition of HMG-CoA reductase results in reduced levels of cholesterol which is a precursor in the biosynthesis of steroid hormones and thus play an important role in the maintenance of pregnancy, parturition, lactation and maternal behavior [14,15,16]. In addition, cholesterol is required for normal development in mammals both *in utero* as well as during the postnatal period [17,18].

In our study, we have selected three readily available variety of statins – pravastatin, atorvastatin and simvastatin. The dosing suspensions of each different statin were prepared in distilled water. Since atorvastatin and simvastatin are lipophilic in nature, their solubility in water is very less. So we have selected a much higher dose for our experimental rat model than the recommended daily human dose (20-80 mg/day) in order to ensure that the concentration required for the effect is reached. Since all statins are not equal and have different therapeutic doses, so we have applied three different dosages in our study. Among the three different statins employed in the present study, simvastatin, in particular, emerged as the most active statin towards inducing fetotoxicity. We have observed that 20 mg/kg/day simvastatin exerted total interceptive effect leading to loss of all post–implantation swelling and producing no live or dead embryos. So the rest of the experiments were conducted with simvastatin alone. Supplementation with a progestin alone is not sufficient to maintain pregnancy. Therefore, co-administration of hydroxyprogesterone caproate along with simvastatin could not stop fetal wastage in rats. But the embryos escaped the interceptive effects of simvastatin under the influence of concomitant administration of exogenous mevalonic acid which is an intermediate in the cholesterol biosynthetic pathway. These results indicate that the toxic effects of simvastatin were related to its mode of action and not to any intrinsic toxicity of the molecule itself.

The majority of statins are developmentally toxic to rats [19]. Developmental toxicity induced by HMG-CoA reductase inihibitor was prevented in *vitro* or *in vivo* by mevalonic acid administration but not by cholesterol or a specific inhibitor of cholesterol synthesis as reported earlier [20,21,22]. An earlier report had suggested that mevalonic acid supplementation along with perinatally administered fluvastatin, prevented maternal toxicity, mortality and cardiac myopathy and the adverse maternal effects were observed with fluvastatin due to exaggerated pharmacologic activity which ultimately led to inhibition of the conversion of HMG-CoA to mevalonate [20]. It has also been found that co-administration of mevalonic acid prevented lovastatin-induced lesions in the gastric mucosa in another study [23]. Since simvastatin is reported to act through the same mechanism of action as fluvastatin, it is likely that inhibition of HMG-CoA reductase was also responsible for simvastatin-induced maternal toxicity. Our study depicted significant drop in plasma steroid levels in simvastatin-treated rats. Mevalonic acid contributed in minimising reduction in estradiol and progesterone levels. Alteration of maternal levels of steroids synthesized from cholesterol could potentially play a role in developmental toxicity although that role has not yet been elucidated.

The present study showed striking post-implantation loss in the highest dosed (20 mg/kg/day) group of simvastatin-treated rats. Occurrence of fetal resorption, as depicted from the histological study suggested that interruption of pregnancy occurred at late gestational phase and indicated the pregnancy-terminating potential of simvastatin. Embryonal resorption could be due to modifications of uterine lining function or maternal toxicity which consequently could induce early resorption and may be studied in future.

Conclusion

This is the first pilot study to assess the adverse effects of statin, as a whole, on pregnancy in rats. Our study suggested a risk to the developing fetus if the mother is exposed to high concentrations of statin during pregnancy. This study demonstrated that the treatment with different statins adversely affected the implantation process in rats, although, the mechanisms causing these effect(s) need to be explored further to highlight the mode of action of statins in pregnancy.

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