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Comparative Study on HDL-Cholesterol Raising Effects of Atorvastatin and Dehydrolovastatin

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ABSTRACT

HDL-cholesterol raising effect of atorvastatin was compared to that of dehydrolovastatin in an 8-week study in 5 equally-membered groups of pre-acclimatized Sprague-Dawley rats. The first and second groups were normal and hyperlipidemic control, while the others were treated with atorvastatin 14.4 mg, dehydrolovastatin 7.2 mg, and dehydrolovastatin 14.4 mg, per 200 g of mouse body weight per day, respectively. Slightly better effect than atorvastatin's could only be achieved by dehydrolovastatin with the same dose.

Keywords: cholesterol, HDL, atorvastatin, dehydrolovastatin

INTRODUCTION

Cholesterol (Figure 1) is an organic compound which is essential for ion transport in cell membrane (Yeagle, 1989; Bastiaanse *et al.*, 1997). It is also the material for biosynthesis of bile (Russel, 2009). It is synthesized mainly in the liver and distributed via the bloodstream (Ott and Lachance, 1981). Since cholesterol is insoluble in water, it is transported in a lipoprotein matrix, e.g. the Low-Density Lipoprotein (LDL) mostly, or High Density Lipoprotein (HDL) (Olson, 1998). In the case of LDL-cholesterol overproduction, termed hypercholesterolemia, the complex is eventually trapped in the wall of blood vessel, creating a plaque, leading to atherosclerosis (Miller *et al.*, 2010). On the contrary, HDL-cholesterol sweeps LDL-cholesterol back to the liver, and its elevated level is associated with low risk of cardiovascular diseases (Lund-Katz and Phillips, 2010).

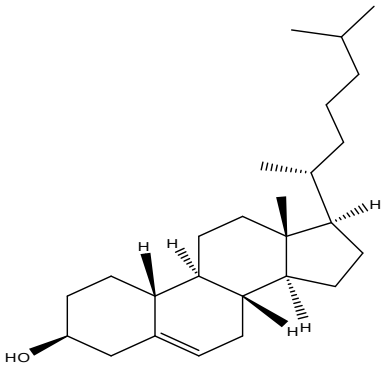

Fig. 1: Cholesterol.

The biosynthesis of cholesterol consists of several steps, in which the reduction of 3-Hydroxy-3-Methylglutaryl– Coenzyme A (HMG-CoA; figure 2) into mevalonate by HMG-CoA reductase is the rate-limiting one (Figure 2) (Goldstein and Brown, 1990). Inhibition of HMG-CoA reductase by compounds that mimic the transition state of the reduction proved to lower the LDL-cholesterol level in blood, hence decreasing the risk of cardiovascular diseases (Bybee *et al.,* 2008; Gotto, 2005). Such inhibitors are: atorvastatin (Bybee *et al.,* 2008), lovastatin (Gotto, 2005), pravastatin(Gotto, 2005), and simvastatin (Gotto, 2005); known as the statin drugs.

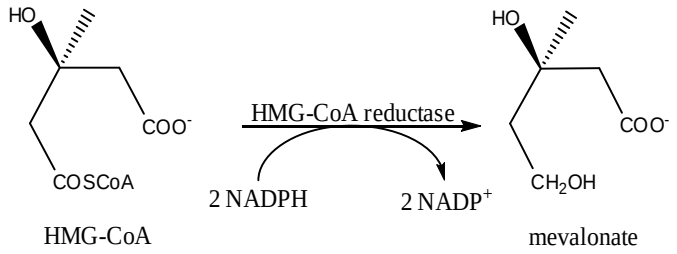

Fig. 2: Reaction catalysed by HMG-CoA reductase.

Atorvastatin (Figure 3) is the best-selling among statins (Pfizer, 2008). A comparative study on the HDL-cholesterol raising effects of atorvastatin and simvastatin have been conducted in humans (Davidson *et al.,* 2003). Obviously, both drugs also raised HDL-cholesterol level at low doses. However, at high doses, atorvastatin progressively lowered it, in contrast to simvastatin.

Meanwhile, our previous experiment in rats (not published yet) revealed that an analog of lovastatin, dehydrolovastatin, raised HDL-cholesterol level better than simvastatin at a low dose, and is safe for short and long term use. Since simvastatin proved to raise HDL-cholesterol level in human more consistently than atorvastatin at high doses, it is of our interest to know whether dehydrolovastatin could raise HDL-cholesterol level in rats better than atorvastatin at a high dose. This investigation would be beneficial for translation into clinical research. Such comparative study is described in the following sections.

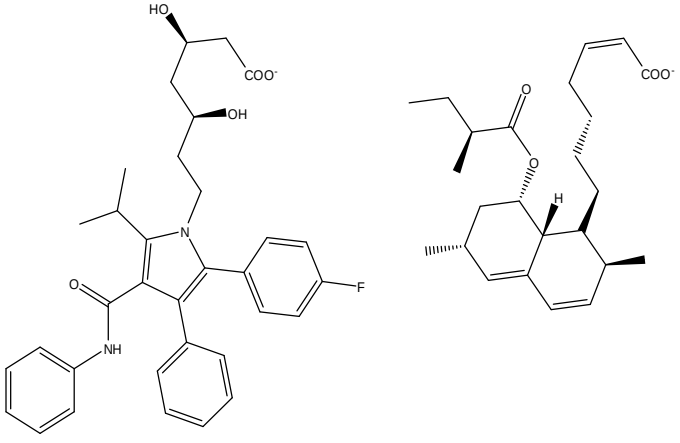

Fig. 3: Atorvastatin and dehydrolovastatin.

MATERIALS AND METHODS

The comparative study on HDL-cholesterol raising effects of atorvastatin and dehydrolovastatin was conducted on 60 eight-week-old male albino rats of Sprague-Dawley strain (150-200 g) which had been selected from our in-house collection and pre-acclimatized for 2 weeks. The rats were randomly grouped into 5 equally-membered groups of daily oral treatment as detailed in Table 1.

Table. 1: Rat groups of treatment.

		Treatment					
No	**Group**	**Standard diet**	**High cholesterol-source diet**	**PTU**	**DLS 7.2 mg / 200 g body weight / day**	**DLS 14.4 mg / 200 g body weight / day**	**AS 14.4 mg / 200 g body weight / day**
1	Normal	√	-	-	-	-	-
2	Hyper-lipidemic control	√	√	√	-	-	-
3	AS	√	√	√	-	-	√
4	DLS 1	√	√	√	√	-	-
5	DLS 2	√	√	√	-	√	-

PTU = propyl thiouracyl. AS = atorvastatin. DLS = dehydrolovastatin.

High cholesterol-source diet included yellow part of eggs (80 %), sucrose (15 %), and animal fat (5 %), and was used to induce hypercholesterolemia exogenously; while PTU (0.01 %) was used to induce hypercholesterolemia endogenously via drinks. Lipitor (Atorvastatin) from Pfizer was used in this experiment.

Dehydrolovastatin was synthesized from lovastatin, and its molecular structure was confirmed by $_{1}$H- and $_{13}$C-NMR. $_{1}$H-NMR (500 MHz, $CDCl_3$): δ 0.84-0.86 (d, 3H), 0.87-0.89 (t, 3H), 1.06-1.07 (d, 3H), 1.09-1.10 (d, 3H), 1.30-1.53 (m, 4H), 1.60-1.71 (m, 3H), 1.90-2.0 (m, 3H), 2.20-2.43 (m, 4H), 4.30-4.35 (m, 1H), 5.37- 5.39 (d, 1H), 5.52 (s, 1H), 5.76-5.79 (dd, 1H), 5.97-5.99 (d, 1H), 6.01-6.02 (d, 1H), 6.84-6.86 (m, 1H). $_{13}$C-NMR (500 MHz, $CDCl_3$): δ 11.89, 14.04, 16.45, 22.99, 24.42, 27.00, 27.62, 29.72, 30.84, 32.58, 32.85, 36.78, 37.44, 41.62, 67.91, 78.71, 121.65, 128.49, 129.86, 131.73, 133.19, 145.01, 164.50, 176.79.

Here, high doses for DLS1 and DLS 2 were based on the assumption that high doses of simvastatin – here taken as gold standard – for human are 40 and 80 mg per day, respectively, whereas human-to-mouse conversion factor is 0.018, and pharmacokinetics factor is 10. After 8 weeks of treatment, each rat was anesthetized. Its chest was sliced open, and 3 drops of heparin were applied onto its heart. Blood was collected carefully from the heart, let at room temperature for 1 hour, then centrifuged at 3,000 rpm and 10 ^{o}C for 10 minutes. Supernatant was stored at -20 ^{o}C and used for measurement. Total cholesterol and HDL-cholesterol level in the supernatant were measured by photometry (CHOD-PAP) according to Henry (1974).

RESULTS AND DISCUSSION

In this experiment, rats were used because of they were immediately available and easy to handle, and the sizes of their

hearts are relatively larger than mice's (Zaragoza, *et al*., 2011), facilitating blood collection from them. Table 2 compiles the total cholesterol and HDL-cholesterol level data of each rat group after 8 weeks of treatment. From this table, we could acknowledge that the high cholesterol-source diet was successful to raise the total cholesterol level and suppress the HDL-cholesterol level in rats, possibly via overproduction of LDL-cholesterol, that the ratio between the total cholesterol and HDL-cholesterol levels raised far beyond 6.0. Total / HDL-cholesterol ratio is one of risk predictors of cardiovascular diseases (Lewington, *et al.*, 2007), while the ratio of 6.0 has been considered as the starting point of high risk of the diseases in humans (Anonymous, 2012).

Treatment with atorvastatin 14.4 mg per 200 g body weight per day did raise HDL-cholesterol level and lower the total /HDL-cholesterol ratio significantly. Slightly better effect than atorvastatin's could only be achieved by dehydrolovastatin with the same high dose.

Table 2. Total cholesterol and HDL-cholesterol levels of each group after treatment

No.	Group	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Ratio
1	Normal	114.08	31.45	3.63
2	Hyperlipidemic control	168.95	14.74	11.46
3	AS	88.81	41.77	2.13
4	DLS 1	95.31	38.33	2.49
5	DLS 2	88.09	42.26	2.08

Ratio = (total cholesterol level) / (HDL-cholesterol level).

We speculated that the slightly differential effects more or less had to do with intestinal absorptions of both compounds in rats, which are functions of their molecular structures. Therefore, we also calculated the ClogPs of both compounds by ChemDraw. The ClogPs for atorvastatin and dehydrolovastatin were 1.50 and 1.96, respectively. This indicates that dehydrolovastatin would have better intestinal absorption in rats than atorvastatin, and correlates positively with the HDL-cholesterol raising effects of both compounds. However, we are more interested to explain these effects by analyzing downstream pathway which is affected by atorvastatin, and probably by dehydrolovastatin as well. In 2008, Seo and colleagues reported that atorvastatin and simvastatin induced the expression of Peroxisome Proliferator-Activated Receptor (PPAR) alpha *in-vitro*, progressively to 10 μM (Seo *et al*., 2008). PPAR alpha is a nuclear receptor that regulates the biosynthesis of HDL-cholesterol, thus has been targeted to raise HDL-cholesterol level (Pal and Pillarisetti, 2007). Based on this report, it is interesting to speculate that the HDL-cholesterol raising effects of atorvastatin were produced via the activation of PPAR alpha. The same hypothesis applies to dehydrolovastatin, since its molecular structure is closely similar to that of simvastatin. These ideas could be a subject for further investigation.

CONCLUSIONS

Dehydrolovastatin could raise HDL-cholesterol level in rats slightly better than atorvastatin at a dose of 14.4 mg per 200 g of rat body weight per day.

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