Simvastatin and Dehydroepiandrosterone Sulfate Effects Against Hypoxic Pulmonary Hypertension Are Not Additive

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Summary

Pulmonary hypertension is a group of disorders characterized by elevated mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance. To test our hypothesis that combining two drugs useful in experimental pulmonary hypertension, statins and dehydroepiandrosterone sulfate (DHEA-S), is more effective than either agent alone, we induced pulmonary hypertension in adult male rats by exposing them to hypoxia (10 %O_2) for 3 weeks. We treated them with simvastatin (60 mg/l) and DHEA-S (100 mg/l) in drinking water, either alone or in combination. Both simvastatin and DHEA-S reduced mPAP (froma mean±s.d. of 34.4±4.4 to 27.6±5.9 and 26.7±4.8 mmHg, respectively), yet their combination was not more effective (26.7±7.9 mmHg). Differences in the degree of oxidative stress (indicated by malondialdehydeplasma concentration), the rate of superoxide production (electron paramagnetic resonance), or blood nitric oxide levels (chemiluminescence) did not explain the lack of additivity of the effect of DHEA-S and simvastatin on pulmonary hypertension. We propose that the main mechanism of both drugs on pulmonary hypertension could be their inhibitory effect on 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, which could explain their lack of additivity.

Key words

Statin • Dehydroepiandrosterone • Pulmonary hypertension • Rats

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Introduction

Pulmonary hypertension is a diverse group of disorders characterized by elevated mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance. Both elevated pulmonary vascular smooth muscle tension and remodeling of the pulmonary vascular wall contribute, to a variable degree, to increased pulmonary vascular resistance. In the absolute majority of patients, pulmonary hypertension is a secondary consequence of some other disease, especially circulatory or respiratory [1]. The prognosis of primary disease is significantly worse if it is complicated by pulmonary hypertension. Although the possibilities of therapy have improved significantly in the last two decades, they still have limited effectiveness and there is a clear need for new therapeutic procedures, including off-label and combination approaches [2].

A good example of an off-label therapy for pulmonary hypertension is the use of statins. Although this group of competitive inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase is used primarily to reduce cardiovascular risks by lowering blood cholesterol levels [3], there is a large body of evidence that they prevent and can reduce pulmonary hypertension in experimental animals [4-7]. The mechanisms of this effect seem to include restoration of

nitric oxide (NO) synthase expression and/or activity reduced in some (but not all [8]) forms of pulmonary hypertension [9,10], decreased plasma concentration of asymmetric dimethylarginine (an endogenous NO synthase inhibitor) [10], and antioxidant activity [11,12] (although a pro-oxidant effect of statins has also been reported [12]). The results with statins in human patients are less conclusive and may depend on the particular type of pulmonary hypertension [13-16]. Therefore, it is worth testing whether combining statins with other therapies could be more effective than statins alone. For example, the combination of statins with sildenafil was shown to be more effective against pulmonary hypertension than either therapy alone in some [17] but not all [18] studies.

Another promising approach to pulmonary hypertension therapy could be the use of dehydroepiandrosterone (DHEA). DHEA is a naturally occurring cholesterol-derived steroid hormone synthesized mainly in the adrenal cortex that serves as a precursor for both estrogens and androgens and has a variety of biological effects of its own [19]. DHEA circulates in the blood mostly in the form of its 3β-sulfate ester (DHEA-sulfate, DHEA-S), which alsomediates many of its effects [20]. DHEA/DHEA-S posess several properties that may be beneficial in pulmonary hypertension, including antioxidant activity [21], stimulation of NO synthesis [22], activation of Ca^{2+}-gated K^+ (K_{Ca}) channels with large conductance (BK_{Ca}) [23] that mediate vasodilation in the pulmonary circulation [24,25], and inhibition of L-type voltage-gated Ca^{2+} channels [26]. Furthermore, DHEA can block superoxide production by alveolar macrophages [27], which is also part of the hypoxic pulmonary hypertension mechanism [28,29]. Because of these properties, and because its exogenous administration is well tolerated in humans, DHEA (or DHEA-S) is attractive as a potential treatment of pulmonary hypertension, especially as plasma levels of DHEA/DHEA-S are reduced in patients with this disease [30]. It has been shown repeatedly that administration of DHEA or DHEA-Spartially prevents and reduces experimental pulmonary hypertension [23,31-33].

The present study was therefore designed to test the hypothesis that a combination therapy with oral statin (simvastatin) and oral DHEA-S will be more effective in preventing chronic hypoxic pulmonary hypertension in rats than either of the treatments alone. Since orally ingested DHEA is converted to DHEA-S when passing through the intestines and liver [20], we decided to use DHEA-S in drinking water.

Methods

The experiments were carried out according to EU regulations for the use of experimental animals and in accordance with the ARRIVE guidelines. They were approved by the Charles University Second Faculty of Medicine Animal Studies Committee.

All drugs and chemicals were purchased from Sigma-Aldrich (Prague, Czech Republic).

Experimental groups and drug administration

The study utilized adult Wistar rats (~350 g at the beginning of the experiment). For the sake of compatibility with previously published studies with statins and DHEA-S, only males were used. They were randomly assigned to one of five groups - one normoxic control (NC, n=16, kept in room air throughout the experiment) and 4 chronically hypoxic (normobaric 10 % O_2 for 3 weeks). Of those, one group was treated with an inhibitor of HMG-CoA reductase, simvastatin, administered in drinking water at a dose of 60 mg/l, throughout the hypoxic exposure (group HS, n=9). Since simvastatin is not soluble in water, it was first dissolved in a small volume of ethanol and then added to the drinking water to yield a final ethanol concentration of 0.5 %. The dose of simvastatin was calculated using our earlier observation (now confirmed) that an adult male rat in 10 % O_2 drinks about 30 ml/day and there ported effective dose by gavage [7]. Another group received, also in drinking water, DHEA-S at 100 mg/l [31] for the entire duration of the hypoxic exposure (group HD, n=10). The third hypoxic group was treated with a combination of DHEA-S (100 mg/l) and simvastatin (60 mg/l) in drinking water with 0.5 % ethanol (group HDS, n=9). The last group drinking plain water in hypoxia served as hypoxic controls (HC, n=9).

We consider the amount of ethanol consumed with simvastatin negligible, as doses ten times higher are used as a model of moderate alcohol use [34,35]. However, to verify that the differences between the groups were not due to the presence of ethanol in the drinking water of simvastatin-treated rats, parts of the remaining groups also received 0.5 % ethanol (8 of 16 rats in NC, 4 of 10 in HD, and 3 of 9 in HC). Unless otherwise stated, the results were the same with and without ethanol and they were thus pooled for statistical analysis.

Experimental protocol and measurements

After 3 weeks of hypoxia (or equivalent age in NC), rats were anesthetized with thiopental (30 mg/kg of body weight, i.p.). Pulmonary artery pressure was measured in intact chest rats spontaneously breathing room air by pulmonary artery catheterization as previously described [31,36]. The trachea was then accessed through a throat skin incision and used to intubate and ventilate the rat with air (50 breaths/min; peak inspiratory pressure 10 cm H_2O; end-expiratory pressure 0 cm H_2O). The chest was then opened by sternotomy and an ultrasound flow probe (Transonic Systems Inc, Ithaca, NY, USA) was placed on the ascending aorta to measure cardiac output [31]. In some rats, this procedure caused excessive bleeding, so the number of rats for which we have cardiac output values is somewhat lower than that for which we have other variables. Cardiac index was calculated as cardiac output/body weight and pulmonary vascular resistance index (PVRI) as mPAP/cardiac output/body weight. Subsequently, arterial blood samples were collected to measure hematocrit. To assess a possible role of NO alterations, the sum of plasma concentrations of NO and its oxidation products (nitrites and nitrates, NO_x) was measured by chemiluminescence (NOA 280i, Sievers, Boulder, CO, USA) after hot acidic reduction as previously described [37].

Serum samples were also used to determine the concentration of malondialdehyde (MDA) with HPLC [38] as a measure of oxidative stress [39]. Briefly, 0.05 % butylhydroxytoluene, 0.44 M H_3PO_4 and 42 mM thiobarbituric acid were added to samples and standards (1,1,3,3-tetraethoxypropane) of different concentrations. The samples were vortexed and then heated at 100 °C for 1 hour. They were then cooled on ice for 5 minutes and the MDA-thiobarbituric acid complex was extracted into butanol. The tubes were centrifuged for 5 minutes at 10,000 g to form two separate phases. Aliquots were pipetted into vials and measured by HPLC (Jasco, Tokyo, Japan). The analysis was performed on the Agilent Zorbax Eclipse Plus C18 4,6x 250 mm, 5 µm column. The optimal flow rate was set at 1 ml/min, for a mobile phase with a composition of methanol/50 mM KH_2PO_4 (40:60, v/v). The fluorescence detector was set at 515/553 nm (excitation/emission). Evaluation was performed using ChromNAV software (Jasco).

Electron paramagnetic resonance (EPR) using 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine hydrochloride (CMH, 10 µM) as a superoxide (O_2^-) detecting spin probe was applied to measure the superoxide production rate in fresh blood [40]. Samples were prepared by adding 5 µMdiethyldithiocarbamate, 25 µM desferroxamine, and 10 µM CMH (Noxygen, Elzach, Germany) and 5 µl of fresh blood in 50 µl of Krebs-Hepes buffer. The samples were placed in airtight glass capillaries and the spectra were recorded in an EPR spectrometer with a temperature-controlled resonator (Escan, Bruker Corp., Billerica, MA, USA). The EPR settings for the CMH spin label were center field 3455 G, sweep width 10 G, frequency 9.7690 GHz, microwave power 23.89 mW, and modulation amplitude 2.93 G. Spectra were recorded over 10 min.

After obtaining all samples, the heart was dissected and weighed in parts in the fresh state. The weight of the right ventricle relative to body weight and to the sum of left ventricle plus septum weight was used as a measure of right ventricular hypertrophy.

Statistical analysis

All differences between the groups were analyzed using 1-way ANOVA followed (if significant) by Fischer´s least significant difference post hoc test using the Prism 9 software (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was preselected to reject a null hypothesis of no difference in all cases. The results are reported as means ± s.d.

Results

All rats assigned to the experimental groups survived till the end of the study with no obvious problems. Compared to normoxic controls, all rats exposed to chronic hypoxia had reduced body weight, but there were no differences in body weight among the treatments, indicating similar water (and thus drug) intake in all hypoxic groups (Table 1). Hematocrit was increased similarly in all hypoxic groups (Table 2), indicating that the effects of therapy on pulmonary vascular resistance and mPAP were not caused by changes in blood viscosity.

As expected, the increase in mPAP caused by chronic hypoxia was reduced (approximately by one half) by the treatment with DHEA-S alone. Similarly, simvastatin treatment alone also significantly reduced mPAP compared to hypoxic controls; the mPAP reducing effect of statin treatment was about the same as that of DHEA-S (Fig. 1a). Contrary to our hypothesis, simultaneous treatment with both drugs (DHEA-S +

statin) did not result in any additional reduction in mPAP (Fig. 1a). Cardiac output and cardiac index did not differ among the groups (Table 2). However, PVRI, significantly elevated in hypoxic controls, did not significantly differ from normoxic controls in any of the treated groups (HD, HS and HDS) and was significantly lower in both groups treated with simvastatin (HS and HDS) than in hypoxic controls (Fig. 1b).

The weight of the left ventricle plus the septum relative to body weight was similar in all groups (Table 1). The weight of the right ventricle relative to body weight, as well as relative to the left ventricle plus septum weight, was increased similarly in all groups exposed to chronic hypoxia (Table 1).

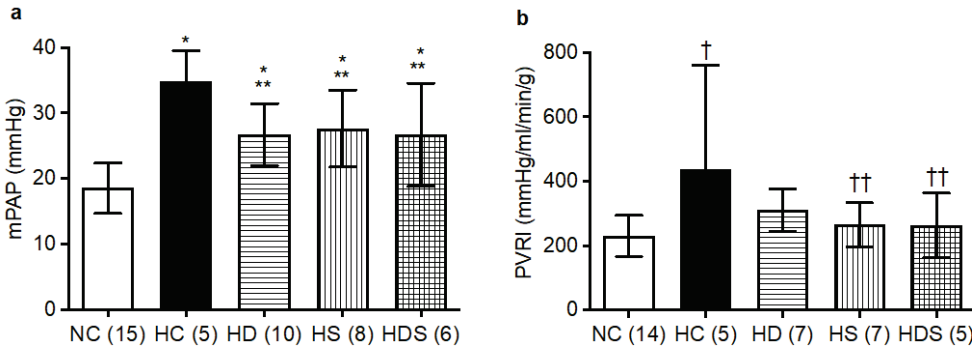

Fig. 1. Mean pulmonary arterial pressure (mPAP, **a**) and pulmonary vascular resistance index (PVRI, **b**), elevated by chronic hypoxia, are reduced by treatment with DHEA-S, simvastatin, and their combination. NC, normoxic control group; HC, hypoxic control group (3 weeks in 10 % O_2); HD, group treated with DHEA-S throughout the 3-weeks exposure to hypoxia; HS, group treated with simvastatin throughout the 3-weeks exposure to hypoxia; HDS, group treated with a combination of simvastatin + DHEA-S throughout the 3-weeks exposure to hypoxia. The columns and lines represent means and s.d., respectively; in parentheses are the *n*s. Differences among the groups not specifically marked are not statistically significant ($p<0.05$, 1-way ANOVA and Fischer´s least significant difference post hoc test). *$p<0.002$ vs. NC, †$p=0.005$ vs. NC, **$p<0.013$ vs. HC, ††$p<0.045$ vs. HC

Table 1. Body and heart ventricles weights

Group (n)	BW (g)	RV (mg)	LV+S (mg)	RV/BW (%)	LV+S/BW (%)	RV/LV+S
NC (16)	446 ± 75	210 ± 21	838 ± 79	0.048 ± 0.007	0.19 ± 0.02	0.251 ± 0.015
HC (9)	327 ± 15*	269 ± 41**	695 ± 51*	0.082 ± 0.011*	0.21 ± 0.01	0.386 ± 0.042*
HD (10)	304 ± 43*	263 ± 56***	638 ± 49*	0.086 ± 0.010*	0.21 ± 0.02	0.409 ± 0.061*
HS (9)	281 ± 23*	239 ± 48	606 ± 50*†	0.085 ± 0.014*	0.22 ± 0.01	0.395 ± 0.073*
HDS (9)	288 ± 33*	241 ± 61	606 ± 6 3*†	0.083 ± 0.015*	0.21 ± 0.01	0.393 ± 0.071*

NC, normoxic control group; HC, hypoxic control group (3 weeks in 10 % O_2); HD, group treated with DHEA-S throughout the 3-weeks exposure to hypoxia; HS, group treated with simvastatin throughout the 3-weeks exposure to hypoxia; HDS, group treated with a combination of simvastatin + DHEA-S throughout the 3-weeks exposure to hypoxia; BW, body weight; RV, right ventrikle weight; LV+S, the sum of the weights of the left ventricle and septum. Data are means ± s.d. Differences among the groups not specifically marked are not statistically significant ($p<0.05$, 1-way ANOVA and Fischer´s least significant difference post hoc test). *$P<0.0001$ vs. NC, **$P<0.005$ vs. NC, ***$P<0.01$ vs. NC

Table 2. Cardiac output, cardiac index, and hematocrit

Group	Cardiac output (ml/min)	Cardiac index (ml/min/kg BW)	Hematocrit (%)
NC	36.9 ± 9.7 (n=14)	84 ± 24 (n=14)	51.4 ± 3.4 (n=16)
HC	36.8 ± 21.0 (n=6)	113 ± 65 (n=6)	63.7 ± 3.2* (n=9)
HD	27.3 ± 6.5 (n=7)	91 ± 17 (n=7)	59.6 ± 6.0 (n=8)
HS	29.0 ± 6.7 (n=8)	103 ± 17 (n=8)	62.3 ± 3.8* (n=9)
HDS	27.0 ± 8.0 (n=7)	92 ± 22 (n=7)	62.4 ± 4.3* (n=9)

Group abbreviations as in Table 1. Data are means ± s.d. Differences among the groups not specifically marked are not statistically significant ($p<0.05$, 1-way ANOVA and Fischer´s least significant difference post hoc test). *$P<0.0001$ vs. NC

DHEA-S alone or simvastatin alone. In rats treated with the DHEA-S + simvastatin combination, the NO_x values Plasma NO_x concentration was significantly elevated by chronic hypoxia. The values were similar in hypoxic controls and in rats treated in hypoxia with did not differ significantly (p=0.064) from those in normoxic controls, but they also did not differ significantly (p=0.313) from the hypoxic controls (Fig. 2).

Plasma MDA concentration, a marker of oxidative stress, was one of the few variables in our study affected by the solvent that the rats drank (water vs. 0.5 % ethanol). MDA levels were significantly higher in animals drinking this weak ethanol solution compared to otherwise identically treated groups drinking water. This was so in normoxic controls and, to a lesser extent, in rats treated with DHEA-S in hypoxia. The same trend existed also in the hypoxic controls, where, however, we were able to measure only two rats drinking ethanol solution, so in this case the data are far from conclusive.

For this reason, we made separate statistical comparisons of MDA for rats drinking ethanol solution (with the exclusion of the too small hypoxia-only group) and water. In water-drinking rats, plasma MDA was slightly reduced by chronic hypoxia and restored by DHEA-S treatment (Fig. 3a). In rats drinking water with 0.5 % ethanol, plasma MDA was highly significantly reduced by DHEA-S treatment and even more so in both groups treated with simvastatin (alone or in combination with DHEA-S) compared to normoxic controls. The two simvastatin groups (HS and HDS) did not differ between each other (Fig. 3b).

The rate of superoxide production also appeared to be affected by the solvent consumed by the rats, at least in normoxic controls, where we had sufficient numbers for direct comparison (3471 ± 423 pmol/min/l of blood in water-drinking rats and 4006 ± 304 pmol/min/l in ethanolsolution-drinking rats, p=0.0113). For this reason, we calculated the differences between groups separately for animals drinking each solvent.

In water-drinking rats we found an increased rate of superoxide production in the DHEA-S treated group compared to the normoxic controls group (Fig. 4a). In the rats drinking weak ethanol solution, the rate of superoxide production was higher in the HDS group compared to both the normoxic controls and rats treated in hypoxia with simvastatin alone (Fig. 4b). Taken together, these data seem to indicate that neither chronic hypoxia nor simvastatin treatment alters the rate of superoxide production. DHEA-S treatment, alone or in combination with statin, on the other hand, does elevate this variable.

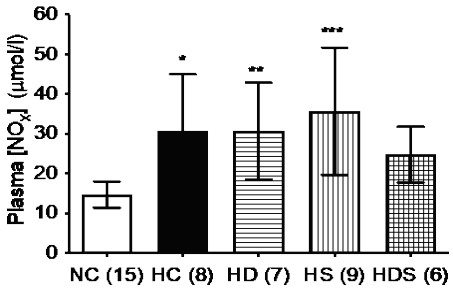

Fig. 2. Plasma concentration of nitric oxide and its oxidation products (NO_x), elevated by chronic hypoxia, is not affected by DHEA-S or simvastatin treatment. When simvastatin and DHEA-S are combined, plasma NO_x no longer differs from normoxic controls. Group abbreviations as in Fig. 1; in parentheses are the *n*s. The columns and lines represent means and s.d., respectively. Differences among the groups not specifically marked are not statistically significant (p<0.05, 1-way ANOVA and Fischer´s least significant difference post hoc test). *p=0.0017 vs. NC, **p=0.0027 vs. NC, ***p<0.0001 vs. NC

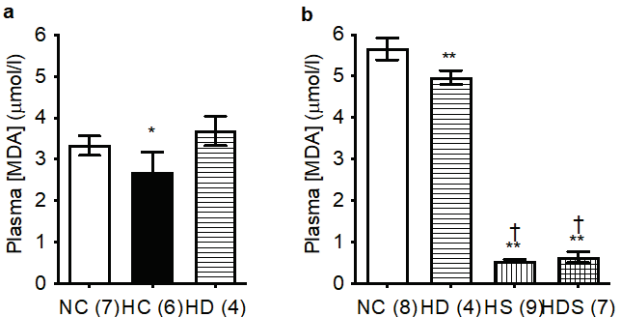

Fig. 3. Simvastatin markedly reduces plasma malondialdehyde (MDA) concentration. **a**) Rats drinking no alcohol. **b**) Rats drinking a weak ethanol solution (0.5 %). Group abbreviations as in Fig. 1; in parentheses are the *n*s. The columns and lines represent means and s.d., respectively. Differences among the groups not specifically marked are not statistically significant (p<0.05, 1-way ANOVA and Fischer´s least significant difference post hoc test). *p<0.01 vs. NC and HD, **p<0.0001 vs. NC, †p<0.0001 vs. HD

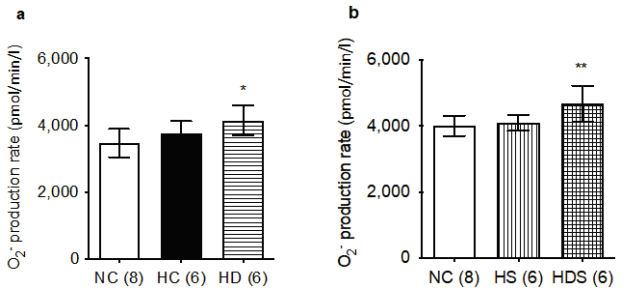

Fig. 4. DHEA-S, but not simvastatin or chronic hypoxia, increase the rate of superoxide (O_2^-) production. **a**) Rats drinking no alcohol. **b**) Rats drinking a weak ethanol solution (0.5 %). Group abbreviations as in Fig. 1; in parentheses are the *n*s. The columns and lines represent means and s.d., respectively. Differences among the groups not specifically marked are not statistically significant (p<0.05, 1-way ANOVA and Fischer´s least significant difference post hoc test). *p=0.008 vs. NC, **p<0.02 vs. NC and HS

Discussion

The main finding of the present study is that treatment of rats with a combination of DHEA-S and simvastatin is about as effective in reducing pulmonary hypertension in a hypoxic rat model as either treatment alone.

Statins are widely used for their cholesterol lowering effect. They competitively inhibit HMG-CoA reductase, the rate-controlling enzyme in the production of cholesterol [3]. In addition to this main effect, statins have a number of other, so-called pleiotropic effects that include antioxidant, antiproliferative, antithrombotic, and anti-inflammatory properties. Following the initial report that statin treatment can reduce experimentally induced pulmonary hypertension [4], a number of studies described the ability of various statins to partly prevent the development of pulmonary hypertension, or reduce an already established one, in several animal models [5-7], although there are also reports of minimal effectiveness of statins against established monocrotaline pulmonary hypertension [41,42].

In human patients, the studies are less conclusive. Three independent meta-analyzes did not find any beneficial effect of statin therapy on pulmonary hypertension from all causes [13-15]. This could be related to the paradox that statins reduce low-density lipoproteincholesterol [43], but low-density lipoprotein cholesterol is already low in patients with pulmonary arterial hypertension, and successful pulmonary hypertension therapy increases these low levels [44]. Nevertheless, analyses focused only on patients with chronic obstructive pulmonary disease (COPD) found reduced pulmonary hypertension in those treated with statins [16]. Our rat model of pulmonary hypertension induced by chronic hypoxia corresponds well with the situation of COPD patients, where the main cause for the development of pulmonary hypertension is their chronically hypoxemic status.

DHEA is an abundant steroid hormone that exists in the blood mainly in the form of its 3β-sulfate ester, DHEA-S, to which it is converted by sulfotransferases especially in the liver and adrenal cortex. Most of the effects of DHEA are mediated by DHEA-S[20]. We and others have shown that DHEA/DHEA-S treatment partly prevents and reverses experimental pulmonary hypertension [23,31-33]. Improved mPAP and pulmonary vascular resistance were then demonstrated in a small group of patients with COPD-related pulmonary hypertension [45]. The beneficial effect of DHEA supplementation corresponds to reduced plasma levels of DHEA-S in patients with pulmonary hypertension [30]. Our results fully confirm the beneficial effect of DHEA/DHEA-S supplementation on pulmonary hypertension.

Somewhat surprisingly, the positive effects of simvastatin, DHEA-S, and their combination on mPAP and PVRI were not reflected by reductions of right ventricle hypertrophy. While traditionally the enlargement of the right ventricle had been considered a simple mechanistic consequence of the afterload increased by the pulmonary hypertension, today it is evident that the relationship between right ventricle size and mPAP is more complex. For example, several long-term studies in humans found no positive effect of epoprostenol treatment on the right ventricular mass despite improvement of pulmonary hypertension [46]. Dissociation between a positive treatment effect on mPAP and no effect on right ventricle hypertrophy has also been reported in animal models [47].

Why are the effects of simvastatin and DHEA-S on pulmonary hypertension not additive? One possibility is that the majority of the beneficial effects on pulmonary hypertension is through the antioxidant activity. The effect of simvastatin on plasma MDA was really profound and DHEA-S could add only little to it, especially as its own effect on MDA was relatively modest. However, this possibility seems unlikely since the effect of simvastatin on pulmonary hypertension was very similar to that of DHEA-S, yet the magnitude of their influence on plasma MDA was quite disparate.

One difference in the mechanism of action between statins and DHEA is their effect on various K channels that control pulmonary arterial vascular smooth muscle cell membrane potential and thus their tension. DHEA activates K_{Ca} channels, specifically the charybdotoxin sensitive BK_{Ca} ($K_{Ca}1.1$) [23]. The possible activation of voltage-gated K^+ (K_V) channels by DHEA was variably confirmed [23] and excluded (with a possible exception of $K_V1.3$) [48]. Statins, on the other hand, are known to stimulate the ATP-sensitive K^+ channels (that play little role in the regulation of pulmonary arterial vascular smooth muscle) [49]. Statins were reported to activate K_V channels in general [50] and to inhibit $K_V1.3$ channels in cancerous T cells [51]. In vascular smooth muscle, $K_V1.3$ upregulation is important for proliferation and migration [52]. Statins' influence on the activity of the BK_{Ca} channels has not been reported.

Thus, DHEA appears to possess a mechanism for pulmonary vasodilation (BK_{Ca} channel activation) that statins lack. This would be expected to result in an additive effect on pulmonary hypertension. The lack of additivity may mean that the participation of the opening of the BK_{Ca} channel in the mechanism of the effect of DHEA on pulmonary hypertension is not essential. Alternatively, statins might activate BK_{Ca} channels indirectly, through their promotion of NO activity, since NO causes pulmonary vasodilation by activating BK_{Ca} channels [25]. Nevertheless, in our experiment, plasma NO_x concentration was not elevated by simvastatin in rats with PH (Fig. 2).

Another possible explanation for the lack of DHEA-S and statin additivity in their effect against pulmonary hypertension could be related to the fact that cholesterol is a precursor of DHEA synthesis. It is thus possible that simvastatin treatment reduced endogenous DHEA (and DHEA-S) production and adding exogenous DHEA-S to the statin therapy merely reconstituted normal levels of DHEA-S rather than increasing them above normal. In fact, a meta-analysis of studies in humans showed that statins decrease DHEA levels in women with polycystic ovary syndrome, although this effect was reported only after atorvastatin therapy, but not simvastatin [53]. However, if DHEA-S and statin affected pulmonary hypertension by different mechanisms, one would still expect the effect of simvastatin alone (presumably with lower DHEA-S levels) to be smaller than its effect with DHEA-S added.

Finally, DHEA can resemble statins in their capacity to inhibit HMG-CoA reductase [54]. This would mean that the reduction of pulmonary hypertension by statins does not belong among their pleiotropic effects unrelated to the inhibition of HMG-CoA reductase. In fact, that is what Girgis *et al.* [6] have concluded from their data showing that attenuation of pulmonary hypertension by simvastatin could be prevented by supplementation with the product of HMG-CoA reductase activity, mevalonate. The mechanisms whereby decreased HMG-CoA reductase activity can lead to less pulmonary hypertension are unknown and deserve further exploration.

One limitation of the present study is that it only used males, but not females. Sex differences in pulmonary vascular physiology and pathophysiology are well established [55]. Normal DHEA-S levels are higher in men than in women [56]. DHEA/DHEA-S levels are lower in men with pulmonary hypertension compared to men without the disease [30]. Cardiovascular benefits of statin therapy seem to be less in women than in men [57]. Therefore, there are grounds to believe that both simvastatin alone and DHEA-S alone could have somewhat different effects in females than in males. However, the focus of the study was the combined effect of the simultaneous administration of both agents, and here it is difficult to imagine argumentation favoring the presence of such a combo effect in females when it was not found in males. Nevertheless, further work on this issue seems warranted.

In conclusion, we confirmed the beneficial effect of statin and DHEA-S treatment on mPAP and PVRI in pulmonary hypertension. We found that combined treatment with both drugs together does not have an additive effect on pulmonary hypertension. One possible explanation is that each of the drugs reduces pulmonary hypertension primarily through their inhibitory effect on HMG-CoA reductase. Statins and DHEA-S can be combined in pulmonary hypertension if needed for other reasons.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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