Development and Preclinical PK/PD Evaluations of Diethylstilbestrol Nanosuspensions in Rats

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ABSTRACT SUMMARY

Subcutaneous nanosuspension (NS) formulations of Diethylstilbestrol (DES) were developed, achieving different particle sizes. The pharmacokinetic profile was assessed after a single subcutaneous dosing and compared with oral and subcutaneous suspension in Sprague Dawley rats. The hepatic exposures along with short and long term toxicities of subcutaneous NS were compared to oral DES. The subcutaneous NS formulations with higher plasma exposures along with lower hepatic exposure and lower toxicity profiles might contribute to maintain the efficacy of DES while improving its safety.

INTRODUCTION

DES is a synthetic non-steroidal estrogen which is used in the treatment of recurrent prostate cancer.

Certain reports suggest that: (1) rates of bone resorption and osteoporosis are less with estrogen therapy than the luteinizing hormone releasing hormone (LH-RH) agonist therapy1, (2) LH-RH agonist therapy has a detrimental effect on the cognitive function which could be reversed by estrogen2, and (3) metabolites of estrogen have significant anti-angiogenic and pro-apoptotic effects2. Thus androgen deprivation without estrogen deprivation certainly warrants further attention2.

Androgen deprivation therapy remains the backbone in managing prostate cancer. DES, is effective in treating both androgen dependent and androgen independent prostate cancer.3-4 But its use has gradually declined due to indirect cardiovascular toxicity caused by inducing the production of coagulation factors in the liver.5

A subcutaneous nanosuspension formulation of DES could possibly reduce hepatic exposure and the cardiovascular toxicity by by-passing the first pass metabolism. In addition, sustaining the release of DES would reduce the frequent dosing of the drug for chronic treatments. DES nanosuspension by subcutaneous delivery is a viable option for potential advantages of: (1) high drug loading and (2) sustained release in vivo.

EXPERIMENTAL METHODS

Two nanosuspension formulations of DES were prepared by the wet milling technique with sizes of 160 (A) and 500 (B) nm, respectively. The DES suspension (C) with a particle size of 2.3 μm and oral suspension (D) in oral suspending vehicle was formulated.

The pharmacokinetic study was performed on Sprague Dawley rats. The animals were administered subcutaneously with a single dose of 7 mg of formulations (A), (B) or (C), using 7 mg DES orally as the reference. For the plasma analysis, the blood samples were collected at 0.04, 0.08, 0.16, 0.33, 0.5, 1, 1.25, 1.5, 2, 2.25, 3, 4, 6 days post dosing for all the groups. The plasma levels of DES were analyzed by a validated LC-MS/MS assay. Peak concentration (Cmax), peak time (Tmax), AUC0-∞ and overall elimination half life (t1/2, β) was determined from the plasma concentration time profiles by using WinNonlin 3.3 software. Plasma fibrinogen (FBG), anti-thrombin III (ATIII) levels (ELISA assay) and rat blood clotting time (CT) for (A), (B) and (D) and sham control groups were monitored for changes in the coagulation cascade (short term toxicity) for 6 days. The hepatic exposures were monitored in SD rats on 0.04, 0.17, 0.5, 1, 2, 4, 6 days (N=4 each time point). Rat livers were perfused and extracted at each time point. The DES extracted from 1 g of liver tissue was quantified by the validated LC-MS/MS assay. The long term toxicity of DES was evaluated with respect to clotting time over a period of 28 days for (A), (B) and (D) groups.

RESULTS AND DISCUSSION

Sizes of the subcutaneous formulations were 162.5 ± 1.5 nm, 492.5 ± 8.3 nm and 2.3 ± 0.2 μm with polydispersities of 0.18 ± 0.01, 0.20 ± 0.03 and 0.37 ± 0.03 for formulations A, B and C (N=3) respectively. The zeta potentials were -27.78 ± 2.11, -22.73 ± 0.58, -16.15 ± 0.62 mV for formulations A, B and C (N=3), respectively.

The pharmacokinetic study demonstrated that sustained plasma DES concentrations were achieved for 6 days from nanosuspension formulations (Figure: 1). Subcutaneous suspension with larger particle size did not show sustained release of DES.
Since DES is practically insoluble in water, the particle size does play a crucial role in the overall release and absorption kinetics of DES when delivered subcutaneously. Larger particle size of the DES suspension accounts for its low solubility and absorption. Thus subcutaneous delivery of DES warrants formulation of smaller particle size suspensions or nanosuspensions to increase the absorption of DES while maintaining its sustained release. The pharmacokinetic profiles of (A), (B) and (D) fitted the 2 compartment model. The elimination half lives were significantly prolonged with 916 and 160 hr for 160 nm and 500 nm, respectively, versus 93 hr for oral suspension. The \( \text{AUC}_{0-\infty}/\text{dose} \) were increased, 948 and 534 ng*hr/ml for 160 nm and 500 nm, respectively, compared to 274 ng*hr/ml of oral suspension with an increase in relative bioavailability of 3.5 for 160 nm and 2 for 500 nm as compared to oral suspension. Plasma ATIII and FBG levels were constant with NS formulations but with oral suspension, significant decreases of 80 % (AT III) and 700 % (FBG) in mean percent change were observed as compared to the sham control. Decrease in AT III indicates a decrease in anti-coagulation mechanism where-as decrease in FBG indicates an excessive utilization of FBG to form fibrin (clot), both resulting in greater clotting potential. Liver \( \text{AUC}_{0-6\text{days}} \), however, decreased significantly with (A) and (B), 87.66 and 45.97 ng*hr/g, respectively, compared to 497.20 ng*hr/g for (D). CT for the (D) group was significantly shorter by 45-55 seconds as compared to those from (A) and (B) for both the short (Figure: 2) and long term toxicity studies. The subcutaneous nanosuspension formulations exhibit higher plasma concentrations with lower hepatic exposures on the same dose basis leading to lower toxicity profiles.

**CONCLUSION**

This study is the first report of successful subcutaneous nanosuspension formulations of DES and their preclinical evaluations for sustained delivery. The nanosuspension formulations resulted in an extended and sustained plasma profiles for 6 days \textit{in vivo}. The subcutaneous nanosuspensions with higher plasma concentration, but lower hepatic exposure resulting in better safety profile, provide a promising route of administration and for maintaining the DES concentrations in the plasma for the treatment of the recurrent metastatic prostate cancer. With a renewed interest in DES, these studies hold great potential for future clinical trials of DES.

**REFERENCES**