Novel combinatorial transdermal drug delivery of alendronate with risedronate for the treatment of osteoporosis

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The presented investigation explores the efficiency of novel transdermal drug delivery system of combination of two drugs i.e. risedronate and alendronate in the treatment of osteoporosis. The nanoparticulate transdermal patch was prepared using PLGA as principle polymer which has been found to be suitable for drug delivery. The novel formulation system was found to be more efficient in lowering and maintaining the plasma calcium at a normal level when compared to a pure drug in a study carried out on an excised rat skin.

Key words: alendronate, risedronate, PLGA, nanoparticles, transdermal patches, osteoporosis

INTRODUCTION

About 10 million women and men in the United States have osteoporosis, characterized mainly by low bone density and deterioration of architecture of bone that increase the risk of bone fractures (Blot et al., 2000; Nelson et al., 2010). Osteoporosis is a vital concern mostly in the geriatric population and about 55% of geriatric population in the United States has been estimated to be affected by it (World Health Organization; Selby, 1996). The two main important characteristics of the Osteoporosis are its adverse effects on microstructure and mass of the bones, and the consequent increased risk of fracture. According to WHO diagnostic criteria osteoporosis is defined by a bone mineral density T-score of −2.5 or less and osteopenia by a low bone mass with bone mineral density T-score between –1 and –2.5 (Waugh et al., 2009). During the last few decades, the development of novel, highly effective therapeutics (e.g., bisphosphonates, strontium ranelate, etc) has remarkably extended the osteoporosis management spectrum. Depending on osteoporosis type, stage and the drugs used for treatment the relative risk of fractures can be maintained between 30% and 60%. In the geriatric population as well as in patients treated with glucocorticoids, frequent falls are an independent risk factor causing both non-vertebral and vertebral fractures, which is correlated to the observed relative lack of efficacy of some bisphosphonates in reducing hip fractures in very old patients with other risk factors besides low bone mineral density (Kim et al., 2006; Handa et al., 2008; Wade et al., 2014; Wells et al., 2008). Bisphosphonates are potent inhibitors of osteoclasts-mediated bone resorption and were found very effective in osteoporosis treatment. This effect is achieved by various mechanisms like promotion of apoptosis of mature osteoclasts leading to decreased bone remodeling, decreased osteoclast progenitors development, decreased osteoclasts recruitment.

MATERIALS AND METHODS

Materials. Alendronate (ALN) and Risedronate (RSD) were supplied by Baoji Guokang Bio-Technology Co., Ltd. (Baoji, Shaanxi, China) Span 80, PLGA (mol. Weight: 30000–60000), Methanol, Liquid paraffin, Trehalose, HPMC, Polyvinyl Alcohol (PVA) were of analytical grade and purchased from Shanghai Chemical Co. (Shanghai, China).

Methods. Preparation of Alendronate (ALD) and Risedronate (RSD) Nanoparticles by high-speed homogenization technique (Allemann et al., 1993).

The ALD and RSD loaded nanoparticles were prepared by high-speed homogenization technique. Span 80 (0.2, 0.4 and 0.6%) solution was prepared in 100 ml of liquid paraffin to produce external phase. PLGA (100 mg, 150 mg and 200 mg) and ALD and RSD (100 mg each) were dissolved in 20 ml of methanol under sonication to produce an internal phase of dispersion. The internal phase of drug solution was added to the external liquid paraffin phase with a syringe at 15000 rpm in a high-speed homogenizer for 10–15 minutes. The whole system was kept under magnetic stirring for 6–8 hours until the complete evaporation of the internal phase (methanol). The resulting dispersion was centrifuged at 10000 rpm for 20 minutes in order to separate the nanoparticles. The supernatant solution was used for determination of the encapsulation efficiency of RSD and ALD. The separated nanoparticles were freeze-dried using trehalose as cryoprotectant and used for further analysis. Prepared RSD and ALD-loaded PLGA nanoparticles were characterized and the optimized nanoparticles were used for the preparation. Composition of alendronate-risedronate nanoparticles (ALN-RSD-NP) is presented in Table 1.

Formulation of patches by Solvent casting evaporation method (Allemann et al., 1993; Dolutabadi et al., 2014).

Solvent casting evaporation method was used for the preparation of transdermal patches in a glass petri dish.
The baking membrane made of PVA was prepared by dissolving it in distilled water at 40°C. This dispersion was poured into a glass petri dish followed by drying in a hot-air oven at 50°C for 8 hours. Optimized ALD and RSD nanoparticles were dispersed in aqueous HPMC solution under continuous stirring. During stirring, DBT was added drop-wise as a plasticizer. This nanoparticle suspension was cast onto the baking membrane and incubated at 50°C for 8 hours in the hot-air oven for drying. The patch was removed from the petri dish and cut into small pieces (1×1 cm), wrapped in aluminum foil and kept in a desiccator for further analysis. Composition of the transdermal patches is presented in Table 2.

### Table 2. Composition of ALN-RSD-NP

| Ingredients                        | F1 | F2 | F3 |
|-----------------------------------|----|----|----|
| Alendronate (mg)                  | 100| 100| 100|
| Risedronate (mg)                  | 100| 100| 100|
| Methanol (ml)                     | 20 | 20 | 20 |
| PLGA (mg)                         | 200| 200| 200|
| Span 80 (ml)                      | 0.2| 0.4| 0.6|
| Liquid paraffin (mg)              | 100| 100| 100|

Characterization of the nanoparticles (Catarina et al., 2006; Bououdina et al., 2013; Amudha et al., 2014; Dolatabadi et al., 2014; Monalisa et al., 2015).

#### Precentage encapsulation efficiency

The resulting nanoparticle dispersion was centrifuged at 10 000 RPM for 20 minutes to separate the nanoparticles. The supernatant solution was used for the determination of encapsulation efficiency of RSD and ALD. The free amount of RSD and ALD in the supernatant was analyzed by HPLC method. 15 μl of the supernatant solution was injected into a chromatograph equipped with a C18 column and UV detector. The mobile phase (80% HPLC water: 20% Acetonitrile) flow rate was 2.5 ml/min; the wavelength was set to 280 nm. The percentage encapsulation efficiency (E.E) of the nanoparticles was calculated according to the following equation:

\[
\text{E.E} = \frac{\text{Total drug-free drug}}{\text{Total drug amount}} \times 100
\]

#### Particle size, PDI (Polydispersity index)

Freeze-dried nanoparticles were dispersed in double-distilled water and sonicated for 20 minutes. Particle size was determined by laser diffraction particle size analyzer and PDI by dynamic light scattering (DLS) using a Zeta Nano ZS instrument.

#### Zeta potential

Zeta potential of RSD-ALN-NP was determined by dynamic light scattering (DLS) using a Zeta Nano ZS instrument.

### RESULTS AND DISCUSSION

The EE was found to be higher in all formulations prepared by high-speed homogenization method. Oil-in-oil solvent evaporation method played a significant role in obtaining higher EE by reducing solubilisation chances of both ALD and RSD in the external liquid paraffin phase. Both drugs are insoluble in the external phase, and the EE was found to be higher in all formulations prepared by high-speed homogenization method. Oil-in-oil solvent evaporation method played a significant role in obtaining higher EE by reducing solubilisation chances of both ALD and RSD in the external liquid paraffin phase. Both drugs are insoluble in the external phase, and

#### Physical characterization

The prepared transdermal patches were inspected visually for color, clarity, smoothness, flexibility, thickness, weight variation and folding endurance.

#### Uniformity of ALD and RSD content

Three patches were dissolved separately in 40 ml of methanol and the solution was filtered to remove the undissolved residues. The drugs content was determined by spectrophotometric measurement at 280 nm.

#### Tensile strength

The tensile strength of the patch was determined for the assessment of the mechanical properties and strength of the patches was determined by using the universal strength testing apparatus (Hounsfield, Slinfold, Horsham, U.K.).

#### In vitro skin permeation study

**In vitro drug release.** The release of ALD and RSD from PLGA nanoparticles was determined by dialysis bag/membrane method. Nanoparticles equivalent to 20 mg ALD and RSD were dispersed in 10 ml of phosphate buffer pH 7.4 in a dialysis tube tied at both ends and placed in 900 ml of buffer solution at 37°C and stirred with a magnetic stirrer. 2 ml of solution was withdrawn at defined time intervals and replenished with fresh solution to maintain sink condition. ALD and RSD release was measured by the HPLC method.

**Characterization of ALD-RSD Transdermal patches** (Khanna et al., 1997; Chandak et al., 2008; Ahmed et al., 2009; Saini et al., 1985).
which improved the EE of nanoparticles. The EE was in the range of 89.90 to 99.80%. The direct relation was observed between EE and the concentration of PLGA. A thick polymeric dispersion was formed due to an increased concentration of PLGA in a fixed volume of methanol that resulted in maximum entrapment of both drugs in the polymeric matrix system. The particle size was determined using laser diffraction particle size analyzer. The particle size was in the range of 243–392 nm. Direct relation was observed between PLGA concentration and particle size. Increase in the amount of polymer concentration resulted in increased particle size. F3 formulation showed PDI of 0.25 which was desired for the nanoparticulate system. Zeta potential of nanoparticles was determined using DLS system and was in the range of –35 to 25 mV. Zeta potential of F3 formulation was 25 mV which indicated a moderate degree of stability due to inner particle repulsion (Cascone et al., 2002). Results are presented in Table 3 below.

In vitro drug release pattern of ALN-RSD is shown in Table 4.

The amount of drug released from polymers largely depended on solvent compatibility, cross-linking as well as on the nature of the polymer. Ionic interactions, mass transfer limitation also contributed to the drug release. The drug release from PLGA nanoparticles was in the range of 75.12–97.13% during 12 h. The formulation batch F3 showed an extended release up to 75.12% which was due to the high concentration of PLGA polymer in it. The strategies like coating of active drugs can greatly help in reducing the toxic effects of some drugs by preventing their direct contact with the tissue (Sahoo et al., 2013).

Characterization of the transdermal patches containing ALN-RSD-NP

Solvent casting method was used to formulate ALN-RSD-NP patch from the optimized batch of formulation F3. Next, the prepared patch was evaluated for thickness, weight variation, folding endurance, drug content uniformity, tensile strength, percent moisture absorption, percent moisture loss, in vitro drug release, and in hypocalcaemia experiment. The results obtained are depicted in Table 5. When evaluated for physical appearance the films were found to be flexible, uniform, and smooth with a transparent surface. Batches of formulations showed uniformity in their weight, thickness, and folding endurance and diameter. The patches showed a thickness from 0.025±0.01 mm to 0.075±0.02 mm. The average weight was found to be between 0.13±0.2 g and 0.17±0.2 g. The folding endurance was of 140–225 with drug content between 94.46–99.47%. The percentage of moisture absorbed ranged between 1.3±0.20 and 2.1±0.21 while moisture loss percentage was between 0.15±1.5 and 3.1±1 (Patel et al. 2009; Sahoo et al., 2013).

Table 3. Characterization of ALN-RSD-NP patches.

| Parameter       | F1      | F2      | F3      |
|-----------------|---------|---------|---------|
| EE (%)          | 89.90   | 96.92   | 99.80   |
| Particle size (nm) | 243    | 359    | 392    |
| PDI             | 0.45    | 0.35    | 0.25    |
| Zeta Potential (mV) | 20     | -35     | 25     |

Table 4. In vitro drug release from ALN-RSD-NP

| Time (h) | F1 ALD | F1 RSD | F2 ALD | F2 RSD | F3 ALD | F3 RSD |
|----------|--------|--------|--------|--------|--------|--------|
| 0        | 0      | 0      | 0      | 0      | 0      | 0      |
| 2        | 20.12  | 18.24  | 17.19  | 15.13  | 12.11  | 13.15  |
| 4        | 43.90  | 42.65  | 31.47  | 42.87  | 32.67  | 36.98  |
| 6        | 62.13  | 67.48  | 47.87  | 62.09  | 54.87  | 53.87  |
| 8        | 73.90  | 89.64  | 68.98  | 87.98  | 69.65  | 61.87  |
| 10       | 97.12  | 98.67  | 98.14  | 90.12  | 79.11  | 75.12  |
| 12       | 97.12  | 98.67  | 98.14  | 90.12  | 79.11  | 75.12  |

Table 5. Physical characterization of the transdermal patches

| Batch | Appearance | Thickness (g) | Folding endurance | Drug content % | % moisture loss | % moisture absorbed |
|-------|------------|--------------|------------------|----------------|----------------|---------------------|
| P1    | ST         | 0.029        | 0.14             | 209            | 93.56          | 0.15                | 1.4                |
| P2    | ST         | 0.062        | 0.17             | 143            | 94.46          | 1.8                 | 2.0                |
| P3    | ST         | 0.075        | 0.13             | 198            | 97.45          | 3.1                 | 1.38               |
| P4    | ST         | 0.048        | 0.18             | 225            | 99.51          | 2.3                 | 3.1                |

T, transparent; S, smooth, ST, smooth transparent; R, rough; HR, hard and rough

Table 6. Assessment of ALN and RSD permeation through rat skin.

| Time (h) | P1 ALD | P1 RSD | P2 ALD | P2 RSD | P3 ALD | P3 RSD | P4 ALD | P4 RSD |
|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0        | 0      | 0      | 0      | 0      | 0      | 0      | 0      | 0      |
| 2        | 13.67  | 16.87  | 19.98  | 21.09  | 19.98  | 24.87  | 15.98  | 10.98  |
| 4        | 21.56  | 28.87  | 24.87  | 32.89  | 32.87  | 38.98  | 25.98  | 29.09  |
| 6        | 38.78  | 39.87  | 40.98  | 50.09  | 45.87  | 45.98  | 39.09  | 31.98  |
| 8        | 50.98  | 56.87  | 62.09  | 71.20  | 53.98  | 54.98  | 47.98  | 43.98  |
| 10       | 76.80  | 69.76  | 78.98  | 89.98  | 71.98  | 76.98  | 59.87  | 57.98  |
| 12       | 97.78  | 89.98  | 95.86  | 90.98  | 97.87  | 87.09  | 70.98  | 68.78  |
The in vitro skin permeation study was carried out and results were plotted as graph as shown in Fig. 1. Patches containing ALN-RSD-NP were subjected to in vitro rat abdominal skin permeation study. The selected formulation was applied to the stratum corneum of the skin of the separated portion of rat abdominal skin. The receptor compartment of the cell was filled with 3 ml of phosphate buffer solution pH 7.4. The permeation of Alendronate was found to be from 70.98% to 89.11% while that of Risedronate was from 68.98% to 92.11% over the period of 12 hours. The results are shown in Table 6. The formulation batch P4 was considered the most optimized due to the highest drug content of 99.51% and prolonged skin permeation (68.98% and 70.68% of Risedronate and Alendronate respectively).

Figure 2 and Fig. 3 shows the comparative plasma concentration of ALD and RSD when administered as pure drugs and in nanoparticulate form. As presented in Table 7, ALD, as well as RSD, administered as pure drug rapidly vanished from blood circulation (BA=1.3 and 1.1 for ALD and RSD respectively) whereas after administration of ALD and RSD in the form of transdermal patches their plasma concentration raised gradually. The bioavailability (BA) was also increased during the first few hours after administration of a dose of 12 mg/kg and 10 mg/kg for ALD and RSD respectively.

Table 7. Data for pharmacokinetic parameters of ALD and RSD in the form of a patch with pure drugs and the nanoparticulate.

| Parameters | Transdermal Patch with Pure drug (ALD and RSD) | Transdermal Patch with NP |
|------------|-----------------------------------------------|--------------------------|
|             | ALD | RSD | ALD | RSD |
| Dose (mg/kg) | 30  | 37  | 12  | 10  |
| AUC (ng.h/mL) | 905 | 897 | 89  | 89  |
| BA (%)     | 1.3 | 1.1 | 6.4 | 7.6 |

The data for plasma calcium concentration profile in hypercalcemia rat model from two different transdermal patch formulations is presented in Table 8. The data suggests the usefulness of the formulation in lowering the plasma calcium level in rats treated with transdermal patches containing CS-ALN-NP while patches containing pure alendronate failed to show a significant decrease in plasma calcium level. In this experiment, plasma calcium concentration was reduced from 15 mg/dl to 8 mg/dl (4 times more) for ALD and from 15 mg/dl to 4 mg/dl for RSD in rat groups treated with transdermal patches with CS-ALN-NP for a week, while the final concentration was only 12 mg/dl (ALD) and 10 mg/dl (RSD) in case of transdermal patches containing the pure drugs (ALD and RSD).

Figure 3. Plasma calcium concentration profile in hypercalcemia rat model treated with two different transdermal patch formulations (Pure drugs and Drugs in nanoparticles) in case of the patches (Pandit et al., 2009). After that, the plasma concentration was steady over 24 hours. The results confirmed almost six to seven-fold increase in bioavailability when drugs were administered in the nanoparticulate form. The pharmacokinetic parameters observed are shown in Table 7.

Hypercalcemia experiment

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Table 8. Plasma calcium concentration profile of ALD-RSD.

| Days | Transdermal Patch with pure drugs (ALD+RSD) | Transdermal Patch with ALD+ RSD NP |
|------|--------------------------------------------|----------------------------------|
| 0    | 20                                         | 20                               |
| 1    | 20                                         | 18                               |
| 2    | 19                                         | 15                               |
| 3    | 16                                         | 10                               |
| 4    | 10                                         | 7                                |
| 5    | 11                                         | 7                                |
| 6    | 11                                         | 7                                |
| 7    | 11                                         | 3                                |
resorption and lead to preventive and therapeutic effects in the treatment of osteoporosis and hypercalcaemia (Pandit et al., 2009).

CONCLUSION

The transdermal patches of ALN-RSD–NP were formulated successfully for anti-osteoporotic treatment. The proposed formulation showed reliable bioavailability which was six to seven times higher than that of pure drugs. The plasma calcium level was maintained by formulations containing ALN-RSD–NP which found to be useful in controlling the osteoporosis. These findings such as enhanced skin permeation, bioavailability and the lower plasma calcium level after transdermal delivery suggest that this is a promising approach for the treatment of osteoporosis.

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