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Melatonin Loaded Biodegradable Nano-Particles and Osteoporosis: A Mini Review

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Abstract **Review Article**

Melatonin loaded PLA-Nano particles are nowadays important in biological system for it's biodegradable nature for targetted drug delivery. Hence, aim of the study is to note applicability and toxicity of Mel-PLA-Nano particles in osteoprosis. Different concentrations of melatonin and PLA were prepared by dissolving in dichloromethane (DCM). The final dried nano-paticles were used for structural analysis by SEM, TEM, FTIR. Toxicity and immunological impact of nano-particles were evaluated on rats; control and nano-particle treated, (n=5/group) for 7 days. Afterwards animals were sacrificed and blood, liver and kidney were collected. A fraction of blood was processed for TLC, DLC and % LC, remaining was centrifuged at 3000x g at 4°C for 30 min. Seperated plasma was used for measurements of IL-2, IL-6, TNF-α, IFN-γ, IL-1β, urea, creatinine and BUN. Both plasma and tissue homogenates were used for AST, ALT, ACP, ALP estimations. We noted signifacntly high ($p < 0.05$) levels of TLC, DLC, %LC and IL-2, TNF-α upon treatment. Rest of the parametrs were found to be significantly low (IL-6, IL-1β, AST, ALT; $p > 0.05$) or un-affected (IFN-γ, ACP and ALP). From our preliminary study we may conclude that we have synthesized Mel-PLA-nano-particles and their effects were non-toxic to animals.

Keywords: Mel-PLA-nano-particles, Characterization, Immune effect, Synthesis, Toxicity.

1. INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced and released by the pineal gland, which has potential to regulate neuro-immune-modulation, anti-cancerous, apoptosis and cell-division by scavenging free radicals [1, 2]. The immune system being an open and multidirectional system has greatly evolved to interact with the endocrine system to combat with the environmental threats. Recently the important role of melatonin has been carried out into the area of neuro-endocrine-immunology in reference to bone deformities [3]. Melatonin receptors are present on the Lymphocytes [4]. This hormone is well established in the regulation of apoptosis and cell-division by enhancing the secretion of mitogens [5]. The effect of melatonin as an anti-oxidant and anti-apoptotic had been proved in a seasonal breeder Funambulus pennanti [6]. The potential clinical benefit of the use of melatonin as an anti-oxidant and anti-apoptotic will be helpful for treatment of many degenerative diseases, such as osteoporosis (a bone degenerative disease) and Alzheimer (a neurodegenerative disease). Considering the important role of melatonin, a proposal has planned to encapsulate the melatonin with biodegradable polymeric nanoparticles and its controlled delivery with desired release kinetics. Biodegradable polymeric nanoparticles have been extensively used with great interest in the area of nano-biotechnology as delivery systems for active molecules and drugs for therapeutic use [7], due to their controlled and sustained-release properties, and biocompatibility with tissue and cells [8, 9]. The current proposal is based on the controlled release of melatonin by encapsulating with biodegradable polymeric nano-particles. The polymeric nanoparticles have the potential to act as a carrier of active molecules and drugs at target sites, protecting them from physiological environment and increase their biological activity. So we have planned a Melatonin and melatonin loaded polymeric biodegradable nano-particles in regulation of osteoporosis and bone deformities in rodents.

1.1. Why polymeric nano-particles?

The polymeric composition (hydrophobicity, surface charge, and biodegradation profile) of the nano-particles, any adjuvant substances, and the associated active molecules and drugs (molecular weight, charge, localization in the nano-spheres by adsorption or incorporation) have a great influence on the active molecules and drugs absorption, bio-distribution pattern

and elimination. The polymeric nano-particles technology used in the recent years has great significance in improving the efficacy of the active molecules and drugs. Nano-particles can be prepared from a variety of biodegradable polymers such as synthetic as well as natural polymers. The choice of materials depends on several factors including (i) size and morphology of the nano-particles (ii) surface charge and permeability of the nano-particle (iii) degree of biodegradability, biocompatibility and cytotoxicity (iv) drug loading and desired release profile.

1.2. Rationale of the study

The main aim of this proposal is to improve the osteoporosis conditions (either post-oestrous or induced) in rodents by developing melatonin loaded polymeric nano-particles and their controlled delivery in rodents as requirement. (i) Osteoporosis is not only an indigenous problem but also it is well prevalent throughout the world. Except for Ca^{+2} supplementation there is no cure is suggested for the treatment of this clinical condition. (ii) Till date, age factor and menopause are regarded as main cause of osteoporosis particularly in humans. But, in this regard, further literature and clinical investigations are completely lacking. (iii) The improvement of osteoporosis by controlled delivery of melatonin loaded nano-particles is the most untouched area of research and during the literature survey we have found very few literatures.

2. CURRENT STATUS OF RESEARCH

2.1. National status

In recent years, rapid scientific and technological advancement have been made in controlled release of active molecules and drugs using biodegradable polymer based nano-particles [10]. To overcome the undesirable side effects, and to protect the efficacy of active molecules and drugs from physiological environment, controlled delivery approach is necessary in order to get optimum therapeutic results. At present, scientists are involved to develop biodegradable polymeric nano-particles by using various biodegradable polymers such as poly-(lactic-co-glycolic acid; PLGA), poly (D,L-lactic acid; PLA), poly (ε-Caprolactone; PCL), chitosan gelatin, and poly-alkyl-cyanoacrylates [11-14] for target delivery of active molecules and drugs related to cancer, diabetes, malaria and other harmful diseases. Our lab is engaged in exploring immunomodulatory role of melatonin considering the bone marrow functioning in rodents and birds [15, 16]. Recently we have published our findings in direction of melatonin's immune protection because of its anti-oxidative and anti-apoptotic action via inhibiting caspase-3 activity in spleen lymphocytes of seasonal breeders under radiation induced stress [17] and also by bone marrow functioning. In India very few groups are involved in bone deformity study [18]. The reasons behind the mechanism of melatonin action in bone anomalies [19] have never been explored at National/International level till date. To the best of our knowledge there is no any information available in India concerning synthesis of melatonin loaded polymeric nano-particle and its delivery approach in comparison to melatonin except for the report of Pandey et al.

2.2. International status

The role of melatonin in execution of different physiological functions like modulation of reproduction [20], immunity [21] is very much well evident. Reports are available on melatonin functioning in bone marrow functioning in modulation of immunity [15]. But, unfortunately, till date a single literature is not available even at international level, regarding the therapeutic use of melatonin or melatonin rich compound in therapeutic use of melatonin in bone deformities study. Further, internationally, very few groups are working in the area of melatonin loaded polymeric nano-particles. Melatonin loaded polysorbate 80-coated Eudragit S100 nanopanicles provided an increase in the m-eitro effect of melatonin against lipid peroxidation in comparison to the melatonin in aqueous solution [22]. Hafner et al. [2009; 23] developed melatonin loaded lecithin/chitosan nano-particles for the transmucosal delivery of melatonin and observed that nano-particles enhance melatonin transport across the epithelial barrier.

3. HYPOTHESIS

It is evident from previous literature [24, 25] that melatonin may impair bone softening by activating Bone Morphogenic Protein 15 (BMP-15). In India (about 50%) and almost 45% population of post-menopausal women of the world are suffering from impaired bone functioning and Fragile Bone Disease [FBD; 26]. This is due to low level of estrogen in circulation which in turn affects the Ca^{+2} absorption from blood. Further, the cesarean deliveries of mothers also lead to impaired bone functions just after menopause. Till date, there is no literature available regarding the proper management of bone fragility in females particularly after menopause except for some commercially available Ca^{+2} supplementation. Taking this background the present dissertation is divided into different parts which are in progress. Since, the studies are underway (unpublished data) we will be providing the glimpses of results in terms of levels of significance wherever necessary.

4. EXPERIMENTAL APPROACHES

4.1. To investigate the possibility of developing and optimization of all conditions for preparations of melatonin loaded polymeric nano-particles

Depending on the physicochemical characteristics of melatonin it is essential to choose the polymer and the method of preparation to achieve an efficient entrapment and controlled delivery of the melatonin. Emulsion polymerization is one of the fastest methods for nano-particle preparation and is readily, scalable. The method is based on the use of on

organic or amt.., continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse micro-emulsion. During preparation of nano-panicles, surfactants or protective soluble polymers may be used to prevent aggregation in the early stages of polymerization. To avoid toxicity, it is necessary to eliminate the organic solvents and surfactants from the prepared nano-panicles. Emulsification-solvent evaporation method may be used for emulsification or the polymer solution into an aqueous phase and evaporation of the organic solvent by inducing polymer precipitation as nano-spheres. The solvent is subsequently, evaporated by increasing the temperature under pressure with continuous stirring. This can be controlled by adjusting the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature.

4.2. Nano-particles preparation

The most suitable biodegradable polymer based nano-particles containing melatonin was prepared by emulsification and solvent evaporation/nano-precipitation method. Briefly, known amounts polymer and melatonin was added into the organic solvent (based on the nature of dispersed phase) and co-dissolved by stirring. The resulting, organic solution was added drop wise into the aqueous phase containing emulsifier under high speed stirring. Nano-particles suspension was collected using an ultra-centrifuge (1, 50, 000 g, 30 min, 4^0C) and washed with distilled water at least three times. Organic solvent was removed from nano-particles suspension by the rotor evaporation technique under reduced pressure at 40^0 C. and finally dried powder of nano-particles obtained. The final dried nano- particles was stored at 4^0 C until further use.

4.2. Nano-particles characterizations

Characterization and evaluation of particle size, morphology, structure and stability of melatonin loaded nano-particles was carried out by using SEM, AFM, FTIR and particle size analyzer. Thermal characterization of pure polymeric nano- particles, melatonin and melatonin loaded nano- particles was carried out using differential scanning calorimetry (DSC).

5. EVALUATION OF TOXICITY OF NANO-PARTICLES IN ANIMAL MODELS

5.1. Administration of melatonin loaded PLA nanoparticles in animals

After synthesis of melatonin loaded PLA nanoparticles the therapeutic efficacy toxicity (if any) of nanoparticles were evaluated in rats. Animals were divided into two groups (control and nanoparticle treated; n=5/group) with ad libitum access to commercially available animal palette and water. In the control group, animals were administrated with normal saline where the treated animals received melatonin loaded PLA nanoparticles. After 7 days, animals were sacrificed and blood and desired tissues (liver and kidney) were collected and were homogenized in respective buffers and were processed for evaluation of different immunological parameters and evaluation of toxicity in terms of Liver Function Test (LFT) and Renal Function Test (RFT).

5.2. Evaluation of toxic impacts of melatonin loaded PLA nanoparticle

Any kind of foreign chemical introduced in body can evoke different physiological side-effects which are mainly of two types. 1. Toxic effects. 2. Immunological side effects. Thus, to evaluate the same we assessed the LFT and RFT in both control and nanoparticle treated groups.

5.2.1. Effect on Liver Function Test (LFT)

Serum Glutamic Oxaoacetate Transaminase (SGOT or Aspertate Aminotransferase; AST) and Serum Glutamic Pyruvic Transaminase (SGPT or Alanine Aminotransferase) are stored in liver under normal conditions. But, upon clinical conditions where the liver is injured (which may be induced due to any kind of drug treatment or due to alcoholic shock) the level of both SGOT and SGPT may be elevated. Thus, assessment of SGOT and SGPT are regarded as the universal markers of liver function. Upon nanoparticle treatment we noted significantly low levels ($p > 0.05$) of SGOT and SGPT as compared to control in plasma and liver. This provided us the first clue that the nanoparticle treatment is not going to affect the most biochemically efficient organ of the body; i.e. the liver. Further, we were interested in evaluating the functioning of another physiologically important organ i.e. the kidney which was assessed by Renal Function Test (RFT).

5.2.2. Effect on Renal Function Test (RFT)

Kidney is one of the most important organs of body actively and effectively participating in detoxification. Any kind of drug administration may hamper renal function as well as glomerular filtration rate (GFR), hence our next aim was to investigate the Renal Function Test (RFT) by assessing plasma urea, Blood Urea Nitrogen (BUN), creatinine, Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP). We noted significantly low levels ($p > 0.05$) of plasma urea, BUN and creatinine levels in nanoparticle treatment groups; however the ACP and ALP levels were found to be unaffected as compared to control. Thus, our results have suggested that the nanoparticle treatment neither had negative impact on liver function nor had negative effect on kidney function. But, any kind of drug treatment (including the nanoparticles) can evoke immunological anomaly in body. Hence, our next aim was to investigate the status of cell mediated and humoral immune parameters upon nanoparticle treatment.

5.3. Evaluation of immunological effects of melatonin loaded PLA nanoparticles

5.3.1. Effect on Cell mediated Immune Parameters

The cell mediated immune parameters are the first marker for any kind of immunogenic or hypersensitivity reaction. To assess the same, we noted significantly high ($p < 0.05$) levels of Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC) and % Lymphocyte Count (%LC) upon nanoparticle treatment as compared to control. These results suggested that, the elevated levels of peripheral cellular components of immunity may be due to some kind of hypersensitivity reaction because of nanoparticle treatment. To investigate the same, we noted humoral immune parameters i.e. cytokines.

5.3.2. Effect on Humoral Immune Parameters

The open circuit of blood immune parameters is chemically coordinated by lymphokines, chemokines and cytokines. Among them, the most effective are the cytokines which are producing the broadest spectrum coordinating a number of immunologically active cells. The cytokines are mainly of three types pro-inflammatory (e.g. IL-2), anti-inflammatory (e.g. IL-6) and switch between pro-and anti-inflammation (e.g. TNF-α). We noted significantly high ($p < 0.05$) levels of IL-2 and TNF-α and significantly low ($p > 0.05$) levels of IL-6 and IL-1β (a cytokine directly associated with plasma cortisol) in nanoparticle treatment group in comparison to control. Further, IFN-γ (a particular cytokine marker for viral infection) was fond unaffected both in control and nanoparticle treatment group.

6. CONCLUSION

From our preliminary study, we may conclude that we have successfully synthesized Melatonin loaded PLA-nanoparticles which are neither having toxic (in terms of LFT and RFT) nor having immunological (in terms of cell mediated and humoral) side effects. Thusa, our preliminary work is based on the synthesis and optimization of melatonin loaded polymeric nano-particles with desired panicle size, increased entrapment efficiency, and controlled release kinetics. The optimized formulation will be applied for treatment of constitutive and induced bone anomalies as well as a special focus on osteoporosis in rodents with relevance to post-menopausal mothers as they are backbone of our family and society.

ABBREVIATIONS

ACP: Acid Phosphatase; AFM: Atomic Force Microscopy; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; DCM: Dichloromethane; DSC: Differential Scanning Calorimetry FTIR: Fourier-transform Infrared Spectroscopy; IL-1β: Interleukin-1 Beta; IL-2: Interleukin-2; IL-6: Interleukin-6; IFN-γ: Interferon Gamma; LFT: Liver Function Test; RFT: Renal Function Test; Mel: Melatonin; PLA: Poly Lactic Acid; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; TNF-α: Tumor Necrosis Factor-Alpha.

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