Toxicity Studies of Oral and Transdermal Formulations of Gentamicin Loaded PLGA Nanoparticles in Animal Model

Bushra Akhtar1, Faqir Muhammad1*, Bilal Aslam1 and Muhammad Kashif Saleemi2

1Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
2Department of Pathology, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: faqir@uaf.edu.pk

INTRODUCTION

Gentamicin, an aminoglycoside, is used for treating various bacterial infections like endocarditis, bone infections, meningitis, urinary tract infections, pneumonia and sepsis. It is favored antibiotic for treatment of nosocomial infections caused by Staphylococcus aureus, Pseudomonas aeruginosa and E.coli (Dorati et al., 2018). It has poor cellular penetration and high water solubility that’s why placed in class III of BCS (British pharmaceutical classification system) (Sabaefard et al., 2016). Gentamicin is protein synthesis inhibitor and acts by binding to 30S ribosomal subunit of bacterial cells. Like other aminoglycosides, gentamicin has low bioavailability and short half-life (Abdelghany et al., 2012). The conventional regimens of gentamicin multiple dosing result in various adverse effects such as hepatotoxicity and nephrotoxicity due to its hostile biodistribution and fast clearance (Dorati et al., 2018). The most convenient site of aminoglycoside injury is epithelial cells of kidney proximal tubules. Patient related factors like underlying renal disease and concurrent administration of nephrotoxic drugs synergistically act with nephrotoxic potential of aminoglycosides. Another very important factor is plasma concentration of gentamicin. There is established relation between kidney injury and fluctuations in serum levels of gentamicin (Jamshidzadeh et al., 2015).

Gentamicin is available in parenteral preparations. It should be administered by intramuscular injection that’s why health care providers must have the access of sharp disposals and safe injection supplies which are usually

A B S T R A C T

Gentamicin is an aminoglycoside and protein synthesis inhibitor. It is available in parenteral and topical forms (for local effects only). We optimized two nanoformulations of gentamicin for achieving therapeutic drug concentration with better patient compliance and studied their safety profiles. Gentamicin loaded poly lactic co glycolic acid nanoparticles (GM-PLGA NPs) were prepared by solvent evaporation method and coated with chitosan for oral delivery. The second formulation was optimized by incorporating GM-PLGA NPs to the baking layer of various polymer ratios for transdermal drug delivery. Both nanoformulations were characterized. Rabbits were divided into 3 groups with 6 animals in each group. Nanof ormulations were administered as single dose/application to rabbits. Blood was drawn daily from jugular vein of rabbits for 5 days post drug administration. Hematological and biochemical parameters were examined. Animals were sacrificed at 5th day of study. Gross morphological changes of vital organs were observed. Liver, kidney and skin samples were harvested, weighed and studied for histopathology. All the results were expressed in terms of Mean ± SE and were statistically analyzed by analysis of variance using Graph pad prism version 6. No gross morphological changes were observed. Both the hematological and biochemical parameters showed non-significant differences (P>0.05). Histopathology revealed no signs of inflammation or necrosis in the treatment groups. Both the formulations were found non-toxic in the experimental animals. So, these might be used as potential carrier for gentamicin delivery in therapeutics.

©2019 PVI. All rights reserved

To Cite This Article: Akhtar B, Muhammad F, Aslam B and Saleemi MK, 2020. Toxicity studies of oral and transdermal formulations of gentamicin loaded PLGA nanoparticles in animal model. Pak Vet J, 40(1): 67-72. http://dx.doi.org/10.29261/pakvetj/2019.121
deficient in low resource settings (Gyawali et al., 2013; Rodgers et al., 2019).

Nanoparticle formulations of antibiotics have numerous advantages over the conventional dosage route. They provide the target specific drug delivery (Appel, 1990) assist the sustained drug release thus reducing the administration frequency. Nanoparticles also reduce systemic toxicity in comparison to the free drug by masking the entrapped drug. Poly lactic co glycolic acid (PLGA) have been used for entrapment of various antibiotics in nanoparticles signifying improved efficacy and drug delivery (Hamidi et al., 2008).

With swift development in nanotechnology, there is emergent interest in application of nanomaterials in various fields like biotechnology, pharmaceutics and medicine (Medina et al., 2007; Linkov et al., 2008). Although literature has cited various reports regarding the toxicity of inorganic nanomaterials (Xiong et al., 2011) yet a little is known, in terms of human health about the safety of nanoparticles used in gene therapy and drug delivery. The risks posed by these nanoparticles to human health must be addressed as they can be administered to human by numerous routes like ingestion, parenteral, inhalational and dermal followed by their distribution to different tissues via systemic circulation (Hu et al., 2011).

In order to solve the issues of limited dosage route and solubility / biodistribution, we optimized two nanoformulations of gentamicin based on biodegradable polymers for oral and transdermal drug delivery. The current study was conducted to figure out the safety profile of these nanoformulations using rabbits as experimental animal.

MATERIALS AND METHODS

Preparation of gentamicin loaded nanoformulations: Two types of gentamicin loaded nanoformulations were prepared. The first formulation was prepared by solvent evaporation method which were then surface modified with varying concentration of chitosan by physical adsorption. This chitosan coated gentamicin nanoparticles (C-GM-PLGA-NPs) were optimized for oral use at the dose level of 10mg/Kg body weight.

The second formulation was a transdermal patch by using gentamicin loaded poly lactic co glycolic acid nanoparticles. They were incorporated to the baking layer of transdermal patch by using eudragit, hydroxy propyl methyl cellulose (HPMC) and polyethylene glycol (PEG). It was meant for topical application optimized at the dose level of 25mg/kg body weight to achieve systemic concentration. Both of these formulations were subjected to safety evaluation in rabbits.

The prepared nanoparticles were characterized for dynamic light scattering, zeta potential, encapsulation efficiency and scanning electron microscopy (in lyophilized form).

Animals and their housing: Study was conducted in healthy rabbits. Animals were kept in animal house of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad with full access to feed and water. All the in vivo experiments were conducted in accordance to the protocols of institutional bioethical committee after obtaining permission vide letter number 2707.

Experimental design and sampling: Animals were divided into three groups (n=6). Group I was control that received normal feed and water. Group II received chitosan modified nanoparticles via oral route while transdermal patch was applied to group III animals. Group II and III received a single dose of assigned nanoformulations. Blood samples were collected from the animals of all groups from the jugular vein. First sample was collected at day 0 (before drug administration) and then after 1st, 2nd, 3rd and 4th day post drug administration. Blood samples from each animal were collected in vacutainers containing ethylene di-amine tetra acetic acid (EDTA) for hematology while without EDTA for serum separation. After 3-4 hours of sample collection, serum was separated by centrifugation at 4500 rpm for 15 minutes. Then serum was carefully removed and stored at -20°C. The serum was further used for biochemical and drug analysis.

Histopathological studies: At the 5th day post drug administration, animals were sacrificed and liver, kidney and skin samples were taken, weighed and stored in 10% formalin solution for histopathological investigations. Tissues were processed through graded ethanol, sectioned, stained with H & E and studied under light microscope (Olympus, Japan) following established protocols in our earlier studies (Muhammad et al., 2008; Hashmi et al., 2013).

Hematological parameters: The hematology was carried out with CBC hematology analyzer (Medonic, Germany). Hematology parameters such as Red blood cells count (RBCs), white blood cells count (WBCs), Haemoglobin, Erythrocyte indices including mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and mean cell volume (MCV) were studied.

Drug analysis: Gentamicin concentration in serum samples was determined with microbiological assay using Bacillus subtilis as test organism following disc agar diffusion method as described in our earlier studies (Muhammad et al., 1999).

Biochemical investigations

Determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST): ALT and AST in serum of rabbits were determined by using commercially available kits (Quimica Clinica Aplicada, S.A., Amposta (Tarragona), Spain). The method used was proposed by International Federation of Clinical Chemistry (IFCC).

Determination of Creatinine and Blood Urea Nitrogen (BUN): Creatinine and BUN were estimated in serum samples by using commercially available kits (Quimica Clinica Aplicada SA, Amposta (Tarragona), Spain).

Statistical analysis: Data values were expressed as Mean ± SE and were statistically analyzed by two way analysis of variance using Graph pad prism version 6.
RESULTS

The nanoparticles were in the size range of 218nm-237nm which is within nano size range. The zeta potential was in range of -37mV to 12mV while encapsulation efficiency was from 89.5% to 93%. Peak serum gentamicin concentration ranged from 3.55 to 4.35 µg/mL for both nano-formulations (Table 1). The morphological nature of nanoparticles was studied by scanning electron microscopy (Fig. 1).

Table 1: Characterization parameters of gentamicin loaded PLGA nanoformulations for oral and transdermal applications to rabbits

| Parameters                        | Values                  |
|-----------------------------------|-------------------------|
| Zeta size                         | 218nm - 237nm           |
| Zeta potential                    | -37mV - 12mV            |
| Encapsulation efficiency          | 89.5% - 93%             |
| Peak serum gentamicin concentration | 3.55 - 4.35 µg/mL      |

Fig. 1: Scanning electron microscopy of nanoparticles in lyophilized form (A) Nanoparticles for oral formulation (B) Nanoparticles for transdermal patch.

DISCUSSION

Biodegradable and polymeric nanoparticles are successfully used for encapsulation of nucleic acid, peptides and proteins. They are also considered as non-immunogenic, non-inflammatory, non-toxic and non-activators of neutrophils. PLGA has been successfully used for the targeted delivery of drugs and various other molecules as well. Since it undergoes hydrolysis and generates biocompatible metabolites i.e. lactic acid and glycolic acid so it is considered as least toxic nanosystem (Bahadad et al., 2016).

The negative value of zeta potential is contributed towards negatively charged PLGA. Potential gradually increases with encapsulation of gentamicin and becomes positive after chitosan coating. Peak serum gentamicin concentrations in rabbits indicated drug absorption for the optimal therapeutic response. Scanning electron microscopy (SEM) of lyophilized form exhibited the porous nature which confers to encapsulation of higher drug concentrations (Fig. 1).

Our study for exploring the toxic potential of both oral NPs and transdermal patch showed no sign of gross morphological changes in the weight of vital organs and/or ratio of vital organs weight to whole body weight (liver & kidney). The ratio remained constant in all the three groups and showed non-significant differences (P>0.05) indicating that there were no significant toxic effects of administered oral C-GM-PLGA NPs and transdermal patch on vital organs weight. Similarly, non-significant differences were observed in hematological parameters of control versus treatment groups of rabbits indicating non-toxic nature of studied nanoformulations.

Biomedical investigations are presented in Figure 4. Different treatment groups exhibited non-significant differences in ALT (Fig. 4A1), AST (Fig. 4A2), creatinine (Fig. 4B1) and BUN (Fig. 4B2) indicating non-toxic potential of tested nanoformulations. Results of histopathological studies are shown in figure 5. In this figure, slides A, B, C refer to the liver tissue of control, oral NPs and transdermal patch treated rabbits respectively, while slides A1, B1, C1 refers to kidney tissue of control, oral NPs and transdermal patch treated rabbits respectively, and slides A2 & B2 refers to the skin of control and transdermal patch treated rabbits. Microscopically liver, kidney and skin tissues were normal and no significant inflammatory or pathological changes were observed indicating nontoxic nature of our nanoformulations.

Results of organ to body weight ratio are shown in terms of average value of ratio ± SE (Fig. 2). There were non-significant differences (P>0.05) among treatment groups. Hematology parameters (RBCs & WBCs count, MCV, MCH and MCHC) are expressed in terms of Mean ± SE (Fig. 3). All the groups showed non-significant difference (P>0.05) in these parameters indicating non-toxic behavior of studied nanoformulations.

Aminoglycosides associated nephrotoxicity refers to the accumulation of small percentage of administered drug into the proximal renal tubular epithelial cells. Gentamicin enters to the tubular cells via endocytosis as it is of polycationic nature and binds to anionic membrane
phospholipids (Quiros et al., 2010). Creatinine and blood urea nitrogen (BUN) were performed as a marker of renal function assessment. The results of creatinine for the control and formulation treated groups are non-significant suggesting that there is no significant change in the renal function of animals as shown in figure 4B1 and 4B2 for creatinine and BUN respectively. It suggests that both the formulations are safe at the administered dose level.

Gentamicin distribution in the form of NPs might be different in comparison to conventional forms. Nano size plays a significant role in drug distribution. Due to the small size and enhanced solubility, it is possible that GM does not accumulate in the tubular cells and thus causes less nephrotoxicity. Further NPs provide a more stable serum drug level. This reduction in serum drug fluctuations leads to less incidences of renal insult by GM (Jamshidzadeh et al., 2015).

Enzyme concentration is useful for diagnosis of abnormalities in liver, heart, kidney and provides very useful information regarding the degree of damage (Salisu et al., 2018). Aminotransferases, serum enzymes, are the intracellular enzymes and usually found at low level in plasma, govern the cellular content release during the process of cellular renewal. Increased levels of AST and ALT refer to hepatic infections following general cell death. It also attributes to the toxic liver damage and severe viral hepatitis (Gbore and Akele, 2010). The results of our study with respect to transaminases (AST & ALT)
Fig. 5: Histopathological examinations of liver (A,B,C), kidney (A1,B1,C1) and skin tissues (A2,B2) of control versus treatment rabbits (n=6) after administration of gentamicin loaded PLGA nanoformulations (H & E, 100x).

are non-significant in comparison to the control group and both the formulations treated groups (oral and transdermal). The results clearly suggested that there was no hepatotoxicity observed at the administered dose level. Both the transaminases values were within normal reference physiological range reported for rabbits (Jones, 1975). The results of study are in compliance with another research which reported no alterations in biochemical parameters of mice after treating them with gentamicin loaded PLGA nanoparticles (Imbuluzqueta et al., 2013).

Biochemical findings were further investigated after performing histopathological studies on liver, kidney and skin tissues of control as well as treated groups. Microscopic examination of liver, kidney and skin tissues of treated rabbits exhibited normal parenchyma with respect to representative control. The study further suggested that these formulations are safe at the administered dose levels.

All the results are comparable with a study in which surface modified (with PEG) gentamicin self nanoemulsifying nanoformulations were used to assess the hematological, biochemical & histopathological alterations in rats after oral use of these nanoformulations. No changes in the net weight of organs (liver and kidney) were observed. Similarly, no changes in hematological or biochemical parameters related to liver were observed. Histopathological findings of liver and kidney did not show any alterations (Umeyor et al., 2017). Another study was aimed to nanoparticle delivery of gentamicin (modified as hydrophobic) for treatment of Brucella melitensis infection in mice. Toxicity studies revealed no alterations in the hematological (blood cells count, hemoglobin, hematocrit, MCV, MCHC) and biochemical parameters (total bilirubin, creatinine and urea). Histology of liver and kidney revealed no toxicity in GM-PLGA NPs treatment group animals while animals who received conventional GM showed mild nephrotoxicity indicating the safety of GM-PLGA NPs (Imbuluzqueta et al., 2013).

Conclusions: It is concluded that optimized NPs based GM formulations are nontoxic in animal model and might be a potential candidate for drug delivery. Since GM is a narrow therapeutic index drug, further controlled trials are required for dose optimization.

Authors contribution: This manuscript is from PhD thesis of BA. BA & FM designed the study. BA conducted the experiments. All authors were involved in data interpretation, write up and final approval of the manuscript. All authors declare no conflict of interest.

REFERENCES
Abdelghany SM, Quinn DJ, Ingram RJ, et al., 2012. Gentamicin-loaded nanoparticles show improved antimicrobial effects towards Pseudomonas aeruginosa infection. Int J Nanomed 7:4053-63.

Appel GB, 1990. Aminoglycoside nephrotoxicity. Am J Med 88:516-520.
Bahadar H, Maqbool F, Niaz K, et al. 2016. Toxicity of nanoparticles and an overview of current experimental models. Iran Biomed J 20:1-11.

Dorati R, Desrizio A, Spalla M, et al. 2018. Sulfate PEG-PLGA/PLGA-H nanoparticles: screening design and antimicrobial effect evaluation toward clinic bacterial isolates. Nanomaterials 8 pii: E37.

Gbore FA and Akele O, 2010. Growth performance, haematology and serum biochemistry of female rabbit (Oryctolagus cuniculus) fed dietary fumonisin. Vet Arhiv 80:431-43.

Hamidi M, Azadi A and Rafei P, 2008. Hydrogel nanoparticles in drug delivery. Adv Drug Deliv Rev 60:1638-49.

Hashmi N, Muhammad F, Javed I, et al. 2013. Nephroprotective effects of Ficus religiosa linn (peepal plant) stem bark against isoniazid and rifampicin induced nephrotoxicity in albino rabbits. Pak Vet J 33:330-34.

Hu YL, Qi W, Han F, et al. 2011. Toxicity evaluation of biodegradable chitosan nanoparticles using a zebrafish embryo model. Int J Nanomed 6:3351-9.

Imbuluzqueta E, Gamazo C, Lana H, et al. 2013. Hydrophobic gentamicin-loaded nanoparticles are effective against Brucella melitensis infection in mice. Antimicrob Agents Chemother 57:3326-33.

Jamshidzadeh A, Heidari R, Mohammadi-Samani S, et al. 2015. A comparison between the nephrotoxic profile of gentamicin and gentamicin nanoparticles in mice. J Biochem Mol Toxicol 29:57-62.

Jones RT. 1975. Normal values for some biochemical constituents in rabbits. Lab Anim 9:143-7.

Linkov I, Satterstrom FK and Corey LM. 2008. Nanotoxicology and nanomedicine: making hard decisions. Nanomed-Nanotechnol 4:167-71.

Medina C, Santos-Martinez MJ, Radomski A, et al. 2007. Nanoparticles: pharmacological and toxicological significance. Br J Pharmacol 150:552-8.

Muhammad F, Riaz A, Akhtar M, et al. 2008. Estimation of atrazine in the stratum corneum and its toxic effects in skin following topical application to rabbits. Toxicol Mech Methods 18:697-703.

Muhammad F, Nawaz M, Khalil T, et al. 1999. Disposition kinetics and dosage of kanamycin in buffalo heifers. Pak Vet J 19:197-9.

 Quiros Y, Vicente-Vicente L, Morales AI, et al. 2010. An integrative overview on the mechanisms underlying the renal tubular cytotoxicity of gentamicin. Toxicol Sci 119:245-56.

Rodgers AM, McCrudden MT, Courtenay AJ, et al. 2019. Control of Klebsiella pneumoniae infection in mice by using dissolving microarray patches containing gentamicin. Antimicrob Agents Chemo 63:e02612-18.

Sabzeifard P, Abdi-ali A, Soudi MR, et al. 2016. Amikacin loaded PLGA nanoparticles against Pseudomonas aeruginosa. Eur J Pharm Sci 93:392-8.

Salisu IB, Shahid AA, Ali Q, et al. 2018. Nutritional assessment of dietary Bt and CP4EPSPS proteins on the serum biochemical changes of rabbits at different developmental stages. Front Nutr 5:49.

Umeyor C, Attama A, Kenechukwu F, et al. 2017. Surface modified Self-Nanoemulsifying Formulations (SNEFs) for oral delivery of gentamicin: in vivo toxicological and pre-clinical chemistry evaluations. Curr Nanomed 7:237-51.

Xiong D, Fang T, Yu L, et al. 2011. Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: acute toxicity, oxidative stress and oxidative damage. Sci Total Environ 409:1444-52.