Research Article

Comparative study of Verapamil, Diltiazem, and Amlodipineon on Gentamicin induced nephrotoxicity in rats

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Abstract

Objectives: Various studies suggest that calcium channel blocker have properties to ameliorate the nephrotoxic effect of gentamicin. Therefore present research was planned to study the effect of Verapamil, Diltiazem and Amlodipine on Gentamicin induced Nephrotoxicity in Rats. **Materials &Methods:** Gentamicin sulphate (100mg/kg) was administered to male wistar rats (positive control) for 9 days to induce nephrotoxicity. Amlodipine besylate (1mg/kg), verapamil hydrochloride (3mg/kg) and diltiazem hydrochloride (40mg/kg) was administered concomitantly with gentamicin in test groups. On the 10thday, 24 hours urine and blood was collected for biochemical analysis and kidneys for histopathology. **Results:** We observed increase in serum creatinine, serum urea and BUN in positive control. Reduction in serum creatinine was observed in all test groups compared to positive control, which was not statistically significant. Urine volume in positive control was significantly lesser and all test groups were comparable to the sham control. We observed increase in urine creatinine and urine albumin levels and decrease in glomerular filteration rate in positive control while, urine creatinine and urine albumin levels were higher in all test groups with substantial increase in glomerular filteration rate compared to positive control. The weight of kidney was significantly increased in positive control, while in all test groups kidney weight was significantly lesser than positive control. On histopathological analysis, degenerative parameters were lesser in all the three test groups compared to positive control, diltiazem showing maximum protection. **Conclusion:** This study concludes that verapamil, diltiazem, and amlodipine exert protection against gentamicin induced nephrotoxicity.

***Keywords*:** Calcium channel blockers, gentamicin, nephrotoxicity

Introduction

The kidney is a vital organ in health and disease. It is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification and excretion of toxic metabolites and drugs (Alarifi et al., 2012; Ferguson et al., 2008). Therefore, the kidney can be considered as a major target organ for exogenous toxicants. The use of nephrotoxic drugs frequently leads to acute kidney injury which may be responsible for increased morbidity and mortality (Tiong et al., 2014).

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Acute renal failure is a complex disorder that occurs in a variety of settings with clinical manifestations ranging from a minimal elevation in serum creatinine to anuric renal failure. It is often under-recognized and is associated with severe consequences (Mehta et al., 2007). Gentamicin has been widely used as an experimental model for acute nephrotoxicity.

The divalent cations Ca^{++} and Mg^{++} are known to competitively inhibit a large number of aminoglycoside-membrane interactions. On the other hand, there are studies suggesting the protective effect of verapamil and dihydropyridines in aminoglycoside induced nephrotoxicity, while diltiazem has shown to aggravate such renal toxicity (Stojiljkovic et al., 2008; Berkels et al., 2005; Li et al., 2009; Ali et al., 2011; Gibey et al., 1991).

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To our best of knowledge, none of the studies have compared effect of calcium channel blockers from different groups on gentamicin induced nephrotoxicity. This study was conducted to know the interaction between gentamicin and various calcium channel blockers which will help to decide selecting drug therapy, if patient is required to take calcium channel blockers along with gentamicin. Therefore aim of the present study is to compare effect of Verapamil, Diltiazem and Amlodipine on gentamicin induced nephrotoxicity in rats.

Material and methods

The study was conducted after obtaining necessary approval from the Institutional Animal Ethics Committee of M.G.M. Institute of Health Sciences and was executed according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Animals

The study was conducted on drug naive male Wistar rats weighing between 200-250 gms. Procurement of rats was from CPCSEA approved licensed vendor and were transported to the animal house facility of MGM Medical College, Navi Mumbai. Rats were housed for initial 2 weeks in the animal house for acclimatization before initiating the study. Rats were maintained at an ambient temperature 25-30°C in 12 hours dark and 12 hours light cycles. Food and water was given ad libitum.

Drugs procurement

Gentamicin sulphate, (Regenta) Regain laboratories, batch no-RG-390; Verapamil HCL (inj VPL), Samartha life sciences, batch no-MB/05/228; Diltiazem HCL (inj dilzem), Torrent pharma, batch no- I222C009; Amlodipinebesylate pure powder obtained from Cipla Ltd.

Induction of Nephrotoxicity

Nephrotoxicity was induced in rats by using Gentamicin in dose of 100mg/kg/day IP for 9days. Rats were divided into following 5 groups.

1- Sham control (N=5) received 1ml saline IP/day

2- Positive control (N=10) received gentamicin in dose as mentioned above

3- Test groups- All test groups received gentamicin in the dose as mentioned above and they were further divided in to 3 subgroups who in addition received one of the test drug for 9 days in doses as mentioned below:

A- (N=10) received Inj verapamil HCL 3mg/kg/d IM

B- (N-10) received Inj diltiazem HCL 40mg/kg/d IM

C- (N-10) received amlodipinebesylate 1mg/kg/d PO

Animal equivalent dose (AED) was calculated for rats using human absolute dose of the test drug (Medhi et al., 2010).

Sample collection for analysis

On the 9th day after giving inj gentamicin with test drug / normal saline, rats were kept in metabolic cages for 24 hrs for urine collection for further analysis. Animals were kept fasted during the urine collection but water was available as libitum.

On the 10th day after 24hrs urine collection, blood was collected after cardiac puncture under pentobarbitone – 30mg/kg anaesthesia (Medhi et al., 2010) for biochemical analysis. Rats were sacrificed after blood collection using overdose of pentobarbitone, abdomen was opened and the kidneys were removed and cleaned. Both the kidneys were weighed separately, examined macroscopically for hemorrhage and change in colour and texture. Then kidneys were preserved in 10% formalin and histopathological grading was done.

Biochemical analysis

Blood samples were analyzed for Serum creatinine, Blood urea, BUN values were derived **(**Urea [mg/dL] / 2.14; Tietz et al., 2014). Urine was analyzed for 24 hours Urine volume, Creatinine, Albumin, Glomerular filteration rate (GFR) was derived (Urine creatinine X Urine volume / Serum creatinine Tietz et al., 2014; Eisner et al., 2010).

Histopathological analysis

Macroscopic examination for hemorrhagic spots, change in colour and texture. Histopathological analysis was done and following parameters were observed and graded (Hur et al., 2013):

- Tubular Necrosis (TN)
- Tubular interstitial Inflammation (TIN)
- Total histopathological score (THS)

All the histopathological findings were scored on 0 to 4, where 0 indicated Absence, 1 indicated mild, 2 indicated moderate, 3 indicated severe, 4 indicated very severe.

Total histopathological score was calculated by following formula:

THS = TD/2 + TN + THS/2

Statistical analysis

Data was collected and entered in Microsoft Excel (version 2007) and statistical analysis was done using Statistical Package for Social Sciences (SPSS) IBM software version 21 and results were obtained. The results were analyzed using ANOVA test for continuous parameters and Chi square test for the discrete parameters. P value of <0.05 was regarded as a statistically significant value.

Results

We have analyzed results in three parts for all parameters.

- Difference between sham control group and positive control group.
- Difference between positive control group and all test groups.
- Difference amongst the test groups.

There was one mortality in positive control group on 8th day of the experiment. No mortality was observed in any other group.

Biochemical analysis

Blood Parameters

The mean Serum creatinine in sham control group was 0.440.11mg/dl and in positive control group, it was increased to 2.731.65mg/dl, the difference was statistically significant (p= 0.01). In test groups, serum creatinine levels were lesser than that of positive control group, but difference was not statistically significant. Amlodipine group showed maximum reduction amongst the test groups.

The mean serum urea in sham control group was 26.4 3.91mg/dl and in positive control group it was increased to 121.44 75.14mg/dl which was statistically significant. (p= 0.017). The mean BUN in sham control group was 12.31.85 mg/dl and in positive control group it was increased to 56.7535.11 mg/dl, which was statistically significant. (p= 0.017).

Amongst the test groups, verapamil group showed increase in blood urea as well as BUN values as compared to positive control group,(p=0.015) whereas no significant changes in amlodipine group and diltiazem group compared to positive control group was observed. However, we found significant difference between verapamil group and diltiazem group for both the values (p= 0.017)(Table 1).

Urinary Parameters

In positive control group, there was significant reduction in 24 hours urine volume when compared to sham control group (p= .008) while urine volume in the three test groups were comparable to that of sham control group. Urine creatinine in sham control group was 60.267.35 mg/dl, where as in positive control it increased to 95.3619.52 mg/dl, which was statistically significant (p< 0.01). There was statistically significant increase in urine creatinine values in all test groups compared to positive control group (p< 0.001), however there was no significant difference amongst the three test groups. Urine albumin, in sham control group was 0.540.11gm/dl and positive control group was 0.780.22 gm/dl, which is statistically significant (p= 0.048). We did not observed any significant difference in urinary albumin values among positive control groups and test groups. GFR of sham control group was 0.930.29 ml/min 100gm of body weight and in positive control group it decreased to 0.210.27 ml/min 100gm of body weight which is statistically significant (p= 0.001). GFR values showed increase in all three test groups when compared to positive control group, which was statistically significant (p= 0.021 for verepamil and diltiazal), however amlodipine group showed maximum increase in GFR amongst all group compared to positive control group (p= 0.014) (Table 2).

Table 1. Biochemical parameters in blood in various groups (Mean SD)

Groups	S. Creatinine (mg/dl)	S. Urea (mg/dl)	BUN (mg/dl)
Sham control	0.44±0.11	26.4±3.91	12.3±1.85
Positive control	2.73±1.65	121.44±75.14	56.75±35.11
Amlodipine	1.44±0.86	118.4±91.03	55.3±42.53
Verapamil	2.43±1.59	217.4±123.79	101.6±57.85
Diltiazem	2.1±0.31	93.5±11.33	43.7±5.30

Table 2. Biochemical parameters in urine in various groups (Mean SD)

Groups	Urine Volume (ml)	U. Creatinine (mg/dl)	U. Albumin (gm/dl)	GFR (ml/min 100gm)
Sham control	22±2.91	60.26±7.35	0.54±0.11	0.93±0.29
Positive control	18.11±1.76	95.36±19.52	0.78±0.22	0.21±0.27
Amlodipine	22±4.55	126±9.17	0.86±0.18	0.69±0.67
Verapamil	20.9±4.79	134.4±6.96	0.81±0.13	0.45±0.34
Diltiazem	22.2±4.71	139.0±10.96	1.0±0.22	0.60±0.17

Kidney weight assessment

The mean weight of the kidneys in sham control group was 0.760.14gms, where as in positive control group it was increased to 5.350.49gms which was statistically significant ($p<0.001$). Mean weight of kidney in amlodipine group was 1.050.16gms, verapamil group 1.130.09 and in diltiazem group it was 1.170.08gms. There was no significant difference in weight of kidney amongst the test groups. All three test groups showed statistically significant decrease in weight of kidney compared to positive control ($p<0.001$ for each of the three groups).

Histopathological analysis

In positive control group, we observed fibrosis and hemorrhage in the kidneys of 7 out of 9 animals. In sham control and test groups we did not observe any such changes. On analysis of histopathological score of all the groups, we observed that there is statistically significant increase in tubular degeneration, tubular inflammation, tubular necrosis and total histopathological score in positive control group when compared to sham control group, which does not show any degeneration, inflammation or necrosis. When we compared positive control group with each individual test group, there was significant reduction in all parameters ($p < 0.001$ in each group). The reduction in score was significantly higher in diltiazem group compared to other test groups, ie with verapamil ($p <0.05$) and amlodipine group ($p < 0.01$) (Table 3).

Discussion

Gentamicin is highly polar in nature it achieves high concentration in renal cortex during elimination which can lead to acute renal insufficiency (Stojiljkovic et al. 2008; Lopez-Novoa et al. 2011). The value of gentamicin in clinical practice would be greatly increased if some agent could protect the kidney from its undesirable side effects. Thus, a therapeutic approach to protect or reverse gentamicin induced renal damage would be a desirable approach.

The main site of gentamicin nephrotoxicity is tubular cytotoxicity, which leads to the death of tubular epithelial cells, mainly within the proximal segment, which is associated with inflammatory component. Gentamicin produces mesangial contraction which results in reduction in GFR. Gentamicin also induces a reduction in renal blood flow, which is the consequence of an increased resistance of the renal vascular bed and a lower perfusion pressure. A lower renal blood flow causes GFR to fall, and sensitizes tubular cell death by reduction of oxygen and ATP availability (Ali et al., 2011). Oxidative stress has also been suggested to have a key role in gentamicin nephrotoxicity. Gentamicin directly increases the production of mitochondrial reactive oxygen species (ROS). It is capable of damaging many cellular molecules including proteins, lipids, and nucleic acids, thus impairing cell function and leading to cell death which may also participate in inflammation (Lopez-Novoa et al., 2011). In in-vivo animal models, reactive oxygen species have been identified as mediators of proximal tubular necrosis and acute renal failure caused by gentamicin. Reactive oxygen species have been consistently demonstrated to be involved in the development of gentamicin-induced acute renal failure (Li et al., 2009).

There are studies suggesting nephroprotective effect of calcium channel blockers in gentamicin induced nephrotoxicity. Possible mechanisms may be blocking the calcium influx to the impaired cells or to the vasodilatory effects which improve the renal blood flow and thereby modulate the development of acute renal insufficiency or the antioxidant activity which can reduce the generation of reactive oxygen species (ROS)(Stojiljkovic et al.2008; Berkels et al. 2005).

Therefore this study was conducted to compare effect of calcium channel blockers on gentamicin induced nephrotoxicity to explore whether they provide nephroprotective effect.

When blood biochemistry was analysed, we observed that the values of the serum creatinine, serum urea and BUN were significantly increased in positive control group when compared with sham control group showing nephrotoxic effects of gentamicin. In all test groups, reduction in serum creatinine was observed when compared to positive control group, but the difference was not statistically significant. Verapamil group showed increase in serum urea and BUN value when compared to positive control group while amlodipine and Diltiazem group showed slight decrease in serum urea and BUN value, none of these changes were statistically significant.

Similar findings were observed, in verapamil treated groups, by (Tamir et al., 1989) and Patil et al. (2014), where they used two different species, (rats and rabbits) while Niemczyk et al. (1991) used oral verapamil. They have observed minimal change in blood creatinine, urea and BUN. Stojiljković et al. (2008) and Akindele et al. (2014) observed significant decrease in serum creatinine, urea and BUN levels, by verapamil and diltiazem in gentamicin induced renal damage. Studies done by Gomez et al., (1989) observed increased serum creatinine levels but no significant change in serum urea and BUN with diltiazem.

Abdel-Rahmanet et al. (2012) and observed significant decrease in serum creatinine while, Li et al. (2009) observed significant reduction in serum creatinine, blood urea and BUN by amlodipine.

In our study maximum reduction in serum creatinine was observed with amlodipine using dose of 1mg/kg/day which

is equivalent to human dose while other studies have used higher dose of 5mg/kg/day (Lopez-Novoa et al., 2011; Abdel-Rahmanet al., 2012), which may be the reason for difference in findings of amlodipine compared to other studies.

Urine volume in positive control group was significantly decreased when compared with sham control group, while urine volume of all the test groups was comparable to the sham control group. Studies done by other investigators have not estimated changes in 24 hrs urinary volume.

While analyzing urinary biochemical findings we observed that urine creatinine and urine albumin values were significantly increased in positive control group when compared with sham control group showing nephrotoxic effects. We observed increase in urine creatinine and urine albumin levels in all the three test groups when compared to the positive control group. Increased urine creatinine probably suggests improved tubular function compared to positive control group, these parameters were not studied by other investigators.

GFR of positive control group showed significant decrease when compared to sham control group showing nephrotoxic effect. All three test groups showed substantial increase in GFR when compared to positive control group, showing nephroprotective effect. Maximum improvement in GFR was observed in amlodipine group which was statistically significant.

The results of amlodipine and diltiazem were consistent with Abdel-Rahmanet et al. (2012), who reported significant improvement in GFR with 5mg amlodipine and Lortholary et al. (1993), who has seen nephroprotective effect in netilimicin, induced nephrotoxicity with 1mg/kg diltiazem.

In the present study, we observed that the weight of kidneys in positive control group increased significantly when compared to sham control group. The increase in weight may be due to the massive necrosis and inflammation. Probable mechanism of renal inflammation may be due to the generation of free radicals as suggested by Berkeles et al. (2005). All test groups have shown significant reduction in weight of kidneys compared to positive control group, which was comparable to sham control group, which is suggestive of significant reduction in inflammation. The findings were in contrast to the study by Akindele et al. (2014), where there was no significant difference in weight of kidneys by verapamil and diltiazem in gentamicin treated animals compared to gentamicin alone.

On histopathological analysis, we observed that there is significant increase in all degenerative parameters ie tubular degeneration, tubular necrosis, tubulointerstitial inflammation and total histopathological score in positive control group when compared to sham control group, suggesting the nephrotoxicity.

Scores of all the parameters were significantly decreased in all the three test groups when compared to positive control group, suggesting nephroprotective action of amlodipine, verapamil and diltiazem. Amongst the test groups, diltiazem showed maximum protection while verapamil showed least protection.

Results of amlodipine group were consistent with previous studies done by Abdel-Rahmanet al. (2012) and Li et al. (2009). Results of diltiazem were in accordance with Lortholary et al. (1993), where diltiazem has shown a protective effect over netilimicin induced nephrotoxicity but differed from Gomez et al. (1989) which suggested that diltiazem aggravates the gentamicin induced nephrotoxicity.

Results of verapamil in our study were similar to studies done by Akindele et al. (2014) but differed from Patil et al. (2014), which suggested verapamil neither protects nor aggravates the nephrotoxicity.

Limitations of the study

- Only one model for the nephrotoxicity has been used.
- Mechanism of gentamicin induced nephrotoxicity was not studied.
- Only one dose of each calcium channel blocker has been tried.

Further studies are required to find exact dose of calcium channel blockers required to produce nephroprotection against gentamicin induced nephrotoxicity.

Conclusion

Experimental model of gentamicin induced nephrotoxicity was standardized. Positive control group showed significant increase in all blood parameters (S. creatinine, S. urea and BUN), Urinary parameters (Ur. Creatinine, Ur. Albumin), kidney weight and all degenerative histopathological parameters. It showed significant decrease in urinary volume and GFR, establishing the nephrotoxic effect of gentamicin.

The obtained results showed, reduction in serum creatinine in the test groups when compared to positive control group, which was not statistically significant where as amlodipine has shown the maximum reduction. Verapamil group showed increase in serum urea and BUN value, where as Amlodipine and Diltiazem group showed slight decrease in serum urea and BUN value when compared to positive control group.

While urine volume of all the test groups was increased compared to positive control group and it was comparable to the sham control group. We observed increase in urine creatinine and urine albumin levels in all the three test groups when compared to the positive control group, diltiazem showing maximum increase. All three test groups showed substantial increase in GFR when compared to positive

control group. Maximum improvement in GFR was observed in amlodipine group.

On histopathological analysis, scores of all the degenerative parameters were significantly decreased in all the three test groups when compared to positive control group, diltiazem showing maximum protection.

The obtained results showed significant nephroprotection with amlodipine, verapamil and diltiazem histopathologically, but nephroprotection, could not be reflected in some of the biochemical parameters, especially by verapamil, which could not be explained.

Conflict of interest: None

References

Abdel-Rahman MN, Kandeel M. 2012. Effect of Amlodipine and Trimetazidine on Gentamicin-Induced Nephrotoxicity in rats. Journal of American Science, 8(6) 328-35.

Akindele AJ, Adeneye AA, Olatoye F, Benebo AS. 2014. Protective effect of selected calcium channel blockers and prednisolone, a phospholipase-A2 inhibitor, against gentamicin and carbon tetrachloride-induced nephrotoxicity. Human & Experimental Toxicology, 33(8):831-46.

Alarifi S, Al-Doaiss A, Alkahtani S, Al-Farraj SA, Al-Eissa MS, Al-Dahmash B, Al-Yahya H, Mubarak M. 2012. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Saudi Journal of Biological Sciences, 19(1):103-10.

Ali B, Al Za'abi M, Blunden G, Nemmar A. 2011. Experimental Gentamicin Nephrotoxicity and Agents that Modify it: A Mini-Review of Recent Research. Basic & Clinical Pharmacology & Toxicology, 109(4):225-32.

Berkels R, Breitenbach T, Bartels H, Taubert D, Rosenkranz A, Klaus W. 2005. Different antioxidative potencies of dihydropyridine calcium channel modulators in various models. Vascul Pharmacology, 42:145–52.

Eisner C, Faulhaber-Walter R, Wang Y, Leelahavanichkul A, Yuen PS, Mizel D, Star RA, Briggs JP, Levine M, Schnermann J. 2010. Major contribution of tubular secretion to creatinine clearance in mice. Kidney International, 77(6):519-26.

Ferguson MA, Vaidya VS, Bonventre JV. 2008. Biomarkers of nephrotoxic acute kidney injury. Toxicology, 245(3):182-93.

Gibey R, Henry JC. 1991. Effects of the calcium-channel blocker diltiazem on gentamicin-induced nephrotoxicity in rats. Pathologie-biologie, 39(5):530-33.

Gomez A, Martos F, Garcia R, Perez B, Cuesta FS. 1989. Diltiazem enhances gentamicin nephrotoxicity in rats. Basic & Clinical Pharmacology & Toxicology, 64(2):190-92

Hur E, Garip A, Camyar A, Ilgun S, Ozisik M, Tuna S, Olukman M, Narli Ozdemir Z, Yildirim Sozmen E, Sen S, Akcicek F. 2013. The effects of vitamin D on gentamicin-induced acute kidney injury in experimental rat model. International Journal of Endocrinology, 2013 1-7.

Li J, Li QX, Xie XF, Ao Y, Tie CR, Song RJ. 2009. Differential roles of dihydropyridine calcium antagonist nifedipine, nitrendipine and amlodipine on gentamicin-induced renal tubular toxicity in rats. European Journal of Pharmacology, 620:97–104.

Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez-Hernandez FJ. 2011. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney International, 79(1):33-45.

Lortholary O, Blanchet F, Nochy D, Heudes D, Seta N, Amirault P, Carbon C. 1993. Effects of diltiazem on netilmicin-induced nephrotoxicity in rabbits. Antimicrobial Agents and Chemotherapy, 37(9):1790-98.

Medhi B, Prakash A. 2010. Practical manual of experimental and clinical pharmacology. New Delhi: Jaypee Brothers Medical Publishers.

Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A. 2007. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Critical care, 11(2):R31.

Niemczyk S, Ludwicka A, Groniowski M, Lewandowski Z, Hasse Z, Wardyn KA. 1991. Nephrotoxicity of aminoglycosides. II. Preventive studies with oral administration of verapamil. Polskie Archiwum Medycyny Wewnetrznej, 85(1):12-18.

Patil AN, Arora T, Desai A, Tripathi CD. 2014. Comparison of the species-sensitive effects of different dosages of calcium and verapamil on gentamicin-induced nephrotoxicity in rats and rabbits. Toxicology International, 21(3):225-231.

Stojiljkovic N, Veljkovic S, Mihailovic D, Stoiljkovic M, Radovanovic D, Ranđelovic P. 2008. The effect of calcium channel blocker verapamil on gentamicin nephrotoxicity in rats. Bosnian Journal of Basic Medical Sciences, 8(2):170-176.

Tamir A, Israeli BA, Aladjem M, Bogin E. 1989. The effect of oral calcium load or verapamil on gentamicin-induced nephrotoxicity. Clinical Chemistry and Laboratory Medicine, 27(11):847-50.

Tietz N, Burtis C, Ashwood E, Bruns D. 2014. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. Philadelphia: Sauders/Elsevier;

Chapter 25, Kidney function tests, 669-707.

Tiong HY, Huang P, Xiong S, Li Y, Vathsala A, Zink D. 2014. Drug-induced nephrotoxicity: clinical impact and preclinical in vitro models. Molecular Pharmaceutics, 11(7):1933-48.