TIME AND CONCENTRATION DEPENDENDENT BIOAVIALABILITY STUDIES OF KETOROLAC TROMETHAMINE PLGA NANOPARTICLES (NPS) USING CaCo-2 CELL LINE

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Abstract

The present investigation is carried out to evaluate the transport efficiency of KNPS and normal ketrolac drug in colon cancer cell line (Caco-2 cells). The transport efficiency was determined in the terms of time dependent and concentration dependent. The transport efficiency of the compounds was also tested in two different directions such as apical and basolateral direction in the presence of drug transporters. In according to our results obtained the present investigation, the intracellular concentration of the KNPS and normal ketrolac drug was increased after 7th hour of incubation time (101.885±0.1 nmol/L mg and 116.44±0.1 nmol/L mg) respectively comparing to the concentration recorded at 30 min (29.11±0.1 nmol/L mg). On the other hand, the concentration of the KNPS and normal ketrolac drug was immensely increased at 250 µg/mL is (188.43±0.5 nmol/L mg) and (93.1±0.02 nmol/L mg) respectively. The transport efficiency of the drug in the presence of MK-571 resulted significant intracellular concentration of KNPS and normal ketrolac drug. Comparing to apical direction of drugs inoculation the basal direction inoculation was found significant in the increase in the concentration of KNPS and normal ketrolac drug.

Key words: Caco-2 cells, MK-571, Apical direction, Basal direction

1.0 Introduction

The bioavailability of a drug product is defined by the U.S. Food and Drug Administration (FDA) as "the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action." The inability to measure medication concentrations at the site of action is due to the fact that this is uncommon in practise. The more frequent definition of bioavailability is "the rate and extent to which the active drug is absorbed from a dosage form and becomes available in the systemic circulation." Typically, when we talk about bioavailability, we're talking about a drug's ability to be absorbed from the GI tract after being administered orally as a dosage form. Any sort of product may be used as the dose form, including a solution, suspension, tablet, capsule, powder, or elixir.

Drug bioavailability has recently attracted attention in both drug development and the first stages of drug discovery. This is a result of the discovery that, rather than ineffectiveness, the majority of candidate medications that failed in clinical trials had issues with ADME (absorption, distribution, metabolism, and excretion) and toxicity. The pharmaceutical industry is working to increase success rates by include ADME and toxicological considerations in drug research from the very beginning. Therefore, it is not unexpected to observe that there have been an increasing number of articles on medication bioavailability for a while.

Chemically, ketorolac is composed of 5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid [1]. Ketorolac is a non-steroidal anti-inflammatory drug that shares chemical similarities with indomethacin. Ketorolac is a racemic combination of [-]S and [+] R enantiomer forms, with the S form possessing analgesic action. The suppression of COX-1 and COX-2, which inhibits the manufacture of prostaglandins and thromboxanes from arachdonic acid, is thought to have anti-inflammatory effects [2-4].Ocular administration of ketorolac lowers the levels of PGE2 in aqueous humour in terms of ophthalmic applications[5].Ketoralac is a well-known nonsteroidal anti-inflammatory drug (NSAID) used to treat Rheumatoid arthritis, inflammation, pain relief, and to lessen aqueous humour and post-operative cancer pain.[6]. A thorough examination of the available literature on ketorolac and empirical data revealed that, in addition to its anti-inflammatory effects, it also demonstrated promising outcomes in the treatment of different malignancies. Ketorolac salt is a recently identified DDX3 inhibitor that can be used to treat oral cancer, according to Sabinda et al.[7].In vitro tests on DDX3 revealed that ketorolac in a hydrogel

formulation combined with rosuvastatin is effective against cancer.2015 research of Khaggeswar et al. in oral squamous cell cancer was reported.[8].

According to Yuna.G, 2015 [9], ketorolac also demonstrated therapeutic benefits in ovarian cancer patients. It is also evident by our previous studies that the Ketrolac nanoparticles exhibited significant antiproliferative activity against SCC-29 cells. The present investigation was carried out to evaluated the bioavailability studies of PLGA-loaded Ketorolac Nanoparticles (KNPS).

2.0 Materials and methods

2.1 Intracellular drug absorption studies

Utilizing ATCC Caco-2 cells, intracellular drug absorption investigations of KNPS were conducted. (Manassas, VA, USA). The Dulbecco's modified Eagle's medium (DMEM), which is supplemented with 1% L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 15% foetal bovine serum, was used to cultivate the Caco-2celle. The culture plates were kept at 37°C in a humidified 5% CO2 environment until the cells reached 80% confluency. For investigations on transport characteristics, the cells are removed and subcultured on fresh culture medium. A method that has been described was used to test the ability of KNPS to go through cells and accumulate there [10]. In a nutshell, Hank's balanced salt solution (HBSS; 5.4 mM KCl, 137 mM NaCl, 0.8 mM MgCl2, 1.3 mM CaCl2, 0.4 mM KH2PO4, 0.3 mM NaH2PO4, and 10 mM HEPES/Tris) was used to incubate Caco-2 cells at 37° C for 3–4 hours. In order to determine how concentration and time affect drug absorption, the incubation medium is then removed, and the cells are fed DMEM along with various concentrations (from 5 to 300 g/mL) of KNPS for varying times (from 10 min to 6 h). For the comparison the intracellular absorption studies have been carried out using normal ketrolac drug.

2.2 Cellular Transport characteristics

Caco-2 cells were seeded at a density of 1 104 cells/cm2 on Millicell Cell Culture Inserts in order to assess the ability of KNPS to cross the cell membrane. (Millipore, Billerica, MA, USA). The cells are exposed to the electrical resistance across the epithelium until it reaches 300 cm2 or slightly higher [11]. When P-gp, MRP, and MRP2 selective inhibitors like verapamil, cyclosporine, and MK 571 are present, the transport efficiency of the KNPS is tested via bilateral cell membrane (basolateral (BL) and apical (AP) direction) transfer. To measure the intracellular concentration of KNPS, the cell lysates were collected by freeze-thawing at the conclusion of the experiment.

3.0 Result and Discussion

3.1Time and concentration dependent Intracellular absorption of KNPS

Different concentrations of KNPS and standard ketrolac were incubated with colon cancer cell line (Caco-2) cells at varied time intervals (Fig. 1A). We noticed that the time dependent intracellular accumulation of KNPS was gradually increased after 7 h and dropped in between 7 and 8^{th} h of incubation (Fig.1A). The concentration of KNPS after 2h of incubation was increased 2.0 fold (58.22±0.1 nmol/L mg) comparing to the KNPS concentration recorded after first 30min (29.11±0.1 nmol/L mg). Whereas, the concentration of normal ketrolac drug after 2h of incubation was increased of about 1.5 fold (33.56±0.5 nmol/L mg) comparing to the concentration of normal ketrolac drug with first 30 min (22.36±1.0 nmol/L mg). However, the concentration of KNPS after 4h and 7h of incubation was increased of about 3.5 and 4.0 fold (101.885±0.1 nmol/L mg and 116.44±0.1 nmol/L mg) respectively. The intracellular concentration of KNPS was dropped after 8^{th} h of incubation to 3.0 fold (38.81±0.5 nmol/L mg) comparing to 116.44±0.1 nmol/L mg recorded at after 7^{th} hour of incubation. On the other hand, the concentration of normal ketrolac drug after 4h and 7h of incubation was increased of about 2.5 and 3.0 fold 55.9 ±0.5 nmol/L mg and 67.08±0.1 nmol/L mg respectively. The intracellular concentration of normal ketrolac drug was dropped after 8^{th} h of incubation to 3.5 fold (19.1±0.3 nmol/L mg) comparing to 67.08±0.1 nmol/L mg (Fig. 1A). This investigation also includes the determination of concentration dependent intracellular concentration of KNPS and normal ketrolac drug (Fig.1B). According to the results obtained, we noted that the intracellular concentration of KNPS at 150µg/mL was raised of about 5 fold (85.65±0.1 nmol/L mg) comparing to concentration recorded at 10µg/mL (17.13±0.5 nmol/L mg). Whereas, the concentration of normal ketrolac drug at 150µg/mL was raised of about 4 fold (53.2±0.1 nmol/L mg) comparing to concentration noted at 10µg/mL (13.3±0.2 nmol/L mg) (Fig.1B). However, the concentration of KNPS at 250µg/mL has been raised of about >11 fold (188.43±0.5 nmol/L mg) comparing to the concentration recorded at 10µg/mL (17.13±0.5 nmol/L mg). On the Other hand, the concentration of normal ketrolac drug at 250µg/mL has been increased of about 7 fold (93.1±0.02 nmol/L mg) comparing to the concentration noted at 10µg/mL (13.3±0.2 nmol/L mg) (Fig.1B). The intracellular concentration of KNPS and normal ketrolac drug was dropped at 300 µg/mL concentration. The concentration of KNPS was dropped to 1.5 fold (62.0±0.1 nmol/L mg) at 300 µg/mL comparing to (93.1±0.02 nmol/L mg) recorded at 250µg/mL. Whereas, the concentration of normal ketrolac drug was

dropped at 300 µg/mL of concentration to 3.0 fold (31.0 ±0.4 nmol/L mg) comparing to (93.1±0.02 nmol/L mg) recorded at 250µg/mL(Fig.1B).

3.2 Transport Characteristics of KNPS and Normal ketrolac drug in the presence of drug transporters

The efficiency of KNPS and normal ketrolac drug to across the Caco-2 cell membrane and subsequent increase the intracellular concentration was tested from apical and basolateral directions. The evaluation carried out in the presence of drug transporters such as, MK 571, verapamil and cyclosporine (Fig 2). The KNPS inoculated via apical and basolateral directions in the presence of MK 571 resulted 233.75±0.3 and 267.11±0.5 nmol/L mg respectively, whereas, normal ketrolac drug resulted 141.31±0.1 and 171.11±0.2 nmol/L mg via apical and basolateral directions respectively in the presence of MK-571 (Fig 2 A). On the other hand, the transport efficiency of the KNPS and normal ketrolac drug in the presence of veramapil drug transporter was found less significant comparing to the transport efficiency of KNPS and normal ketrolac drug in the presence of MK-571(Fig 2B). KNPS inoculated via apical and basolateral directions resulted 138.31±0.1 and 171.09±0.2 nmol/L mg respectively, whereas, normal ketrolac drug resulted 118.10±0.5 and 146.05±0.1 nmol/L mg via apical and basolateral directions respectively (Fig.2B). However the transport efficiency of the KNPS and normal ketrolac drug through the Caco-2 cell membrane in the presence of cyclosporine was found average. KNPS inoculated via apical and basolateral directions resulted 115.01±0.5 and 129.11±0.3 nmol/L mg respectively, whereas, normal ketrolac drug resulted 104.12±0.4 and 120.01±0.1 nmol/L mg via apical and basolateral directions respectively (Fig 2C).

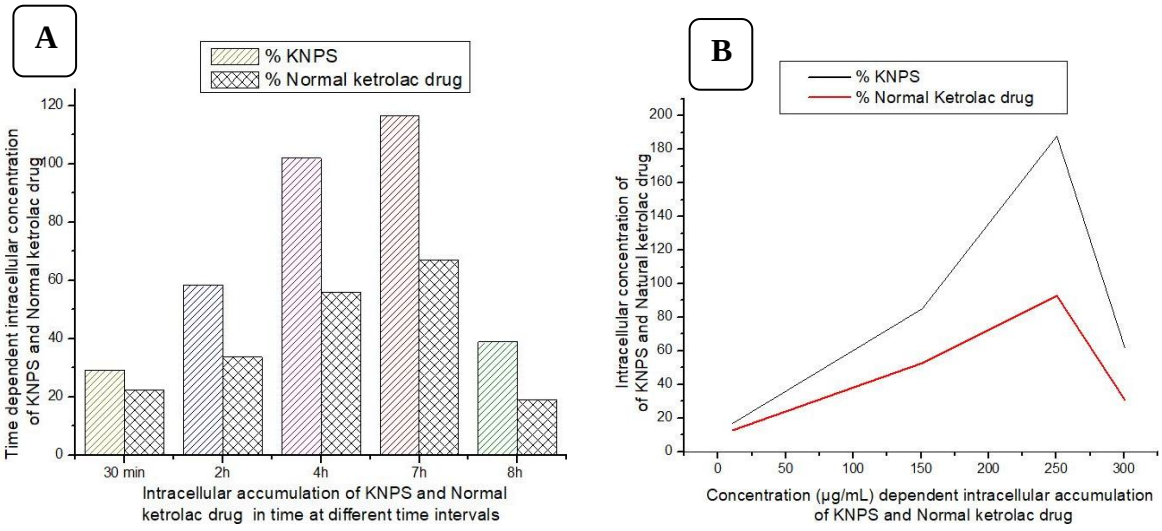

Fig 1 A-Time dependent intracellular accumulation of KNPS and normal ketrolac drug. B- Concentration dependent intracellular accumulation of KNPS and normal

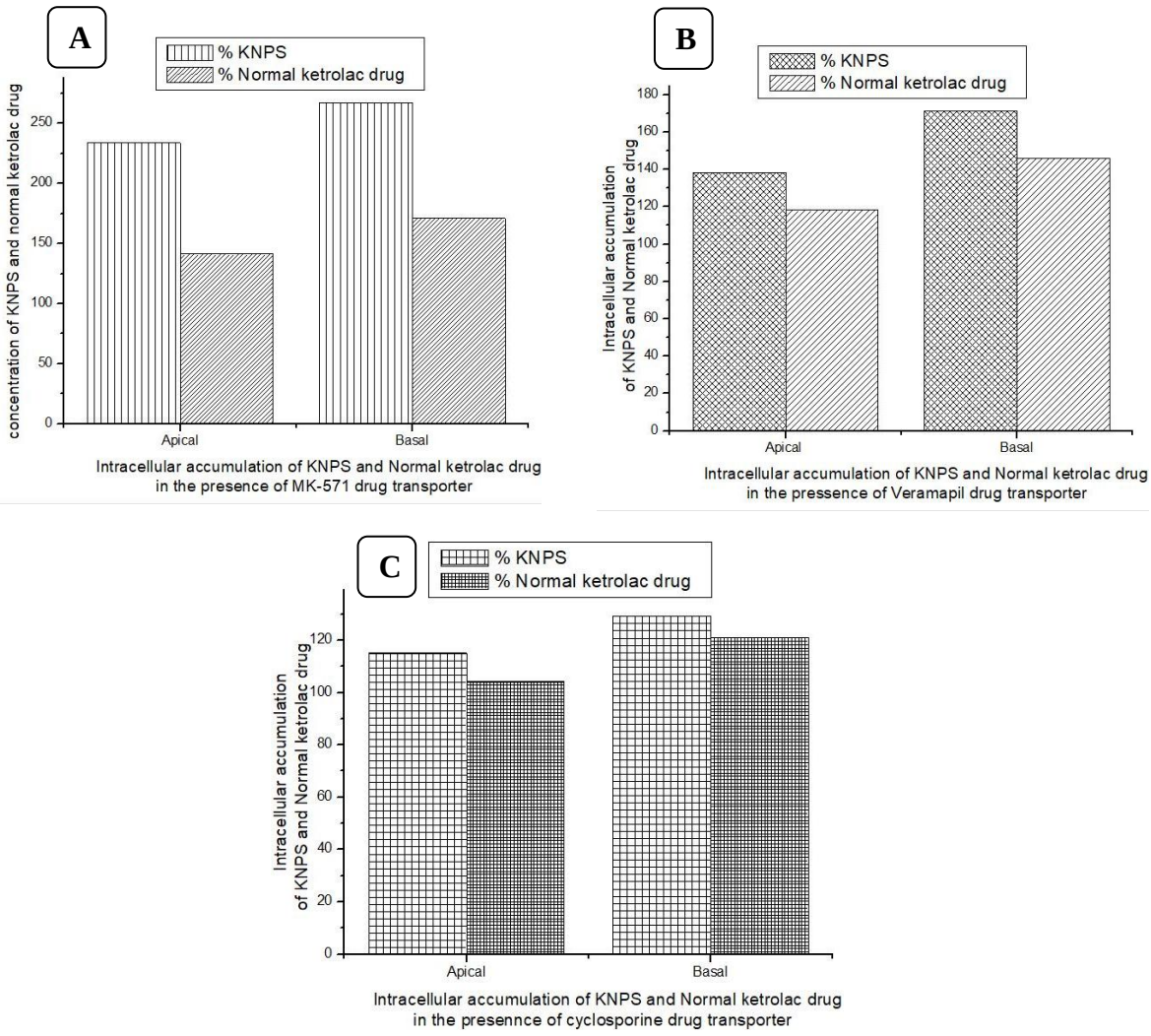

Fig. 2 A-Intracellular accumulation of KNPS and normal ketrolac drug in the presence of MK-571 drug transporter, B- Intracellular accumulation of KNPS and normal ketrolac drug in the presence of Veramapil drug transporter, C-Intracellular accumulation of KNPS and normal ketrolac drug in the presence of Cyclosporine

Conclusion

With reference to the results obtained in this study, here by we conclude that, the bioavailability of the KNPS was found significant comparing to the normal ketrolac drug. Basal direction of drug inoculation was found significant way to increase the intracellular concentration of KNPS comparing to apical direction. However the drug absorption studies are more significant in the presence of drug transporters.

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