



Original Research Article

POTENTIATION OF THE CYTOTOXIC ACTIVITY OF MELOXICAM AGAINST COX-2 NEGATIVE COLON CANCER CELLS BY NANOPARTICULATE ENCAPSULATION

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ABSTRACT

Objective: The aim of the current work was to elucidate whether the nanoencapsulation of meloxicam would allow its exertion of anticancer activity on colon cancer cells despite lacking COX-2 expression.

Methods: Meloxicam nanoemulsion was prepared using the water dilution method, in which oleic acid was chosen as the oily phase, tween 20 as surfactant and ethanol as cosurfactant. The nanoemulsion was characterized in terms of particle size, zeta potential, polydispersity index, morphology, *in vitro* drug release, stability and anticancer activity on HCT-116 colon cancer cells.

Results: It was shown that meloxicam nanoemulsion was successfully prepared with a particle size of 11 nm, polydispersity index of 0.278 and a neutral charge, and it was able to sustain the release of drug for 24 hours with a cumulative percent released of 86%. Its spherical morphology was confirmed using transmission microscopy. No significant changes in particle size, polydispersity or charge were observed upon storage, suggesting the stability of the nanosystem. Moreover, the nanoemulsion exhibit significant cytotoxicity on HCT-116 compared to non-encapsulated meloxicam, suggesting that the prepared nanoemulsion was a successful delivery carrier for meloxicam, which is capable of potentiating its anticancer activity in a non COX-2 dependant manner.

Keywords: Meloxicam, nanoemulsion, colon cancer, HCT-116 cells, COX-2.

INTRODUCTION

Re-profiling of drug substances is currently a hot topic in the pharmaceutical field, in which new therapeutic opportunities are being discovered for already existing drugs [1,2], to minimize the time required for drug discovery and development, in addition to reduction of the associated costs. Meloxicam is a well-known non steroidal anti-inflammatory drug, categorized as selective COX-2 inhibitor. It was recently discovered that meloxicam could act as an antitumor agent, especially in colon cancer [3,4], owing to the involvement of COX-2 in the regulation of colon tumor growth. Therefore, meloxicam was reported to be an effective anticancer drug against colon cancer cells expressing COX-2 such as HCA-7 and Moser-S cells, but was rather ineffective against COX-2 negative cells as HCT-116 [3]. Therefore, in an attempt to increase the effectiveness of meloxicam against non COX-2 expressing cells, its formulation in a nanoparticulate carrier was attempted in the current

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work.

Several nanoparticles were reported to increase the anticancer activity of drugs [5-7], owing to their ability to enhance cellular internalization by virtue of their small size [8]. Among the promising nanoparticles are nanoemulsions, which are composed of oil, water and surfactant/cosurfactant mixture [9-11], and can be prepared as an isotropic extremely small sized (from 10-100 nm) delivery system using the water dilution method (termed microemulsions) [12], and hence can be better uptaken by cancer cells. A water dilutable nanoemulsion based on oleic acid as the oily phase and tween 20 as surfactant was prepared by Deng et al., 2015 [13], and to the authors' knowledge, its efficacy was only tested when loaded with antibiotics [14]. Therefore, in the current manuscript, a very small sized nanoemulsion was prepared and loaded with meloxicam, in order to test its ability to potentiate the cytotoxic activity of meloxicam against non responsive cyclooxygenase negative cell line HCT 116.

MATERIALS AND METHODS

Materials

Oleic acid, tween 20, absolute ethanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, dimethylsulfoxide, MTT dye and dialysis membrane (molecular weight cut off 12000-14000) were purchased from Sigma Aldrich Co., USA. Meloxicam was kindly obtained by Hikma pharmaceutical company, Jordan. Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% trypsin-EDTA were purchased from Lonza, Belgium. HCT-116 cancer cells were obtained from the American type culture collection (ATCC, USA).

Preparation of meloxicam nanoemulsion

The meloxicam-loaded nanoemulsion was prepared using the water titration method [9,10], in which 10 mg meloxicam was dispersed in a mixture composed of 4.1 ml tween 20, 0.28 ml oleic acid and 0.32 ml ethanol, and stirred using a magnetic stirrer. The mixture was titrated up to 10 gram water dropwise till the formation of an oil in water nanoemulsion loaded with meloxicam.

Determination of the particle size, polydispersity index and zeta potential of meloxicam nanoemulsion

The particle size, zeta potential and polydispersity index (PDI) of the prepared meloxicam nanoemulsion was measured using the Zetasizer device (model ZS3600, Malvern, UK).

Morphological examination using transmission electron microscopy

The prepared meloxicam nanoemulsion was visualized using transmission electron microscopy without staining, after being dried on a carbon-coated grid (VERSA 3D, USA).

In vitro release of meloxicam from the prepared nanoemulsion

The release of meloxicam from the nanoemulsion was performed using a dialysis-based method [15-17]. One ml of meloxicam nanoemulsion was placed in a cylinder of a length eight cm and radius five cm, and attached to the shaft of USP dissolution apparatus (Pharma Test, Germany). The shaft was rotated at 50 rpm and 37°C, and the release medium was 200 ml phosphate buffer pH 7.4 containing 2% tween 20 to ensure sink

conditions for meloxicam. Two ml samples were taken from the release medium at definite time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 24 hours), and the amount of meloxicam released was measured spectrophotometrically at wavelength 269 nm (SPUV UV/VIS double beam spectrophotometer, SCO TECH, Germany)

Assessment of the stability of meloxicam nanoemulsion

The properties of the nanoemulsion (particle size, PDI and zeta potential) were re-measured after 3 months storage at room temperature, to assess the stability of the prepared formulation [18].

Evaluation of the cytotoxicity of meloxicam and the nanoemulsion in HCT-116 cancer cell line

HCT-116 cells were grown on RPMI-1640 medium containing 10% inactivated fetal calf serum and 50µg/ml gentamycin at 37°C in a humidified atmosphere with 5% CO₂, and were subcultured twice to three times every week. When properly grown, cells were placed in culture medium at a concentration of 50000 cells per well (Corning® 96-well tissue culture plates) then incubated for twenty four hours. Either meloxicam alone dissolved in 0.5% dimethyl sulfoxide, or meloxicam nanoemulsion was added to the cells at different concentrations, in addition to vehicle controls with media or 0.5 % DMSO. The cell viability was assessed after 24 hour incubation, in which the media was substituted with another fresh media containing MTT dye followed by incubation for 4 hours and further addition of dimethyl sulfoxide. The optical density was measured at 590 nm with a microplate reader (SunRise, TECAN Inc, USA) to calculate cellular viability according to the following equation [17]:

$$\text{Viability \%} = \frac{OD_t}{OD_c} \times 100\%$$

in which OD_t and OD_c is the optical density of the treated and untreated cells respectively. The IC₅₀ value (the concentration causing 50% cellular death) was calculated.

Statistical analysis

Measurements were done in triplicate and reported as mean±S.D. T-test was performed using Graphpad® InStat software, at significance of P≤0.05. The IC₅₀ values were calculated using Graphpad Prism software (San Diego, CA. USA)

RESULTS AND DISCUSSION

Measurement of particle size, PDI and zeta potential of meloxicam nanoemulsion

As shown in Figure 1, the meloxicam nanoemulsion displayed a particle size of 11.01±0.25 nm, a PDI value of 0.278±0.01 and a zeta potential value of 0.08 mV. Owing to the surfactant and cosurfactant content of nanoemulsions prepared by the water dilution method, they are known to exhibit very small particle size [14]. The low polydispersity of the nanoemulsion indicates its homogeneity, and the effective solubilization of meloxicam within the oily phase of the nanoemulsion. The almost neutral charge on the particles of the nanoemulsion is attributed to the non-ionic nature of the surfactant constituting the majority

of the formulation.

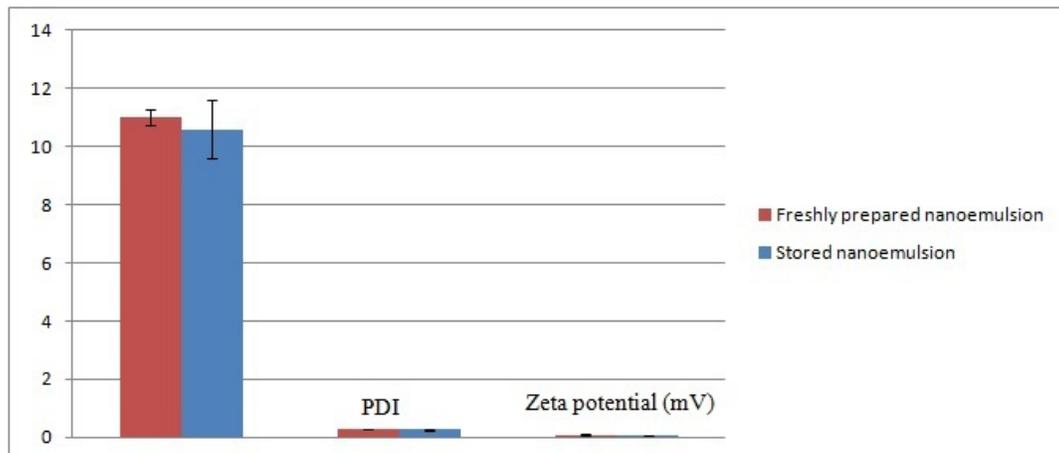


Figure 1: The particle size, PDI and zeta potential values of meloxicam nanoemulsion either freshly prepared or after storage.

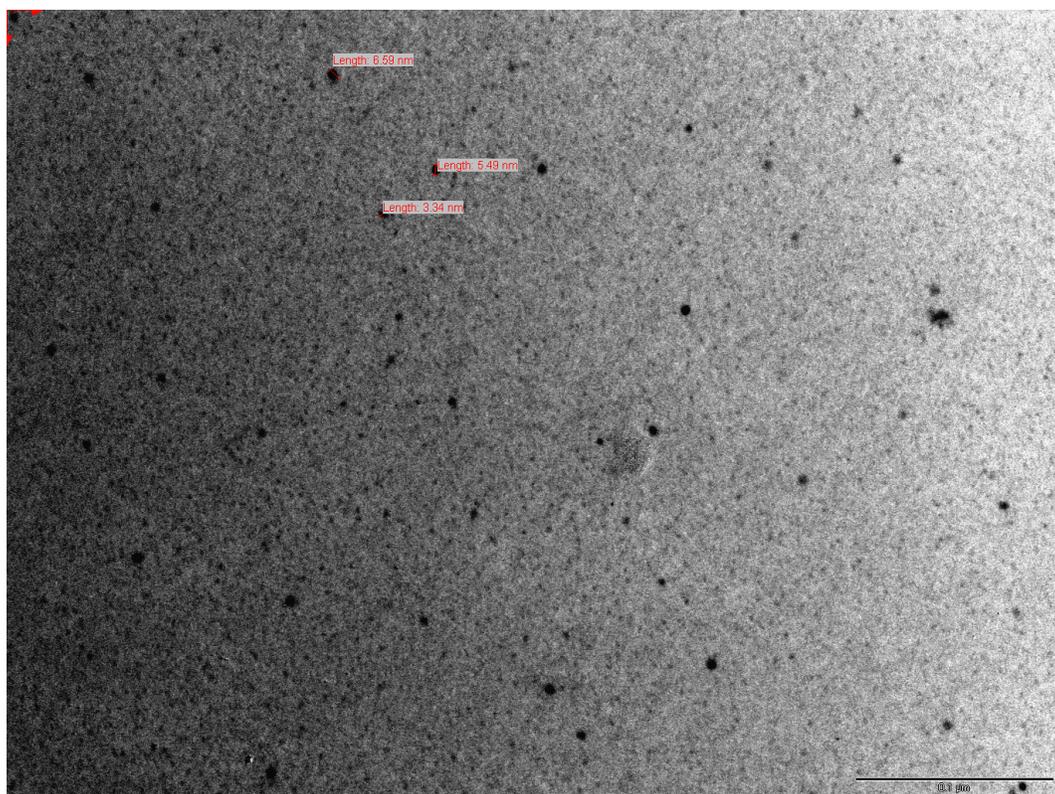


Figure 2: Transmission electron microscopy picture of the prepared meloxicam nanoemulsion.

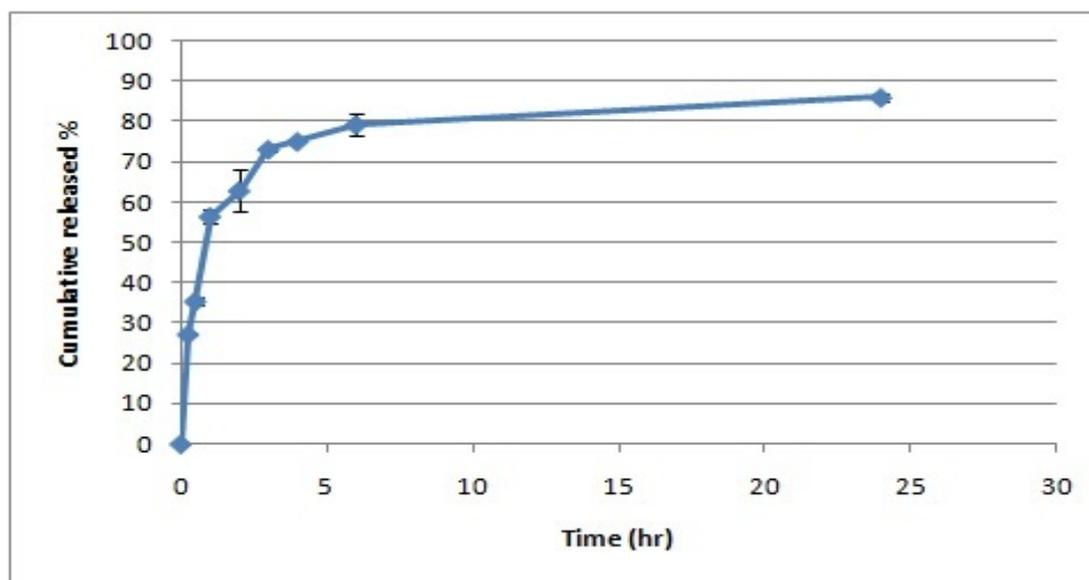


Figure 3: Cumulative percent released of meloxicam for 24 hours from the nanoemulsion formulation.

Morphological examination of the nanoemulsion using transmission electron microscopy

As shown in Figure 2, the meloxicam nanoemulsion displayed homogenous non-aggregated spherical droplets, confirming the small size obtained with the Zetasizer.

***In vitro* release of meloxicam from the nanoemulsion**

As seen from Figure 3, a sustained release of meloxicam was achieved over 24 hours from the nanoemulsion, reaching a percentage of 86% after 24 hours. The sustained release property of the nanoemulsion is advantageous for cancer treatment, since after internalization of the particles in the cancer cells, meloxicam is expected to be released in a continuous manner over time.

Stability of meloxicam nanoemulsion

As shown in Figure 1, no significant changes occurred to the particle size, zeta potential or PDI of the meloxicam nanoemulsion after storage for 3 months ($P > 0.05$), suggesting the stable nature of the prepared nanoemulsion.

Table 1: HCT-116 cancer cell viability percentage obtained as a function of concentration for meloxicam and meloxicam nanoemulsion

Sample conc. ($\mu\text{g/ml}$)	Cell viability % for meloxicam nanoemulsion	Cell viability % for meloxicam
500	2.75 \pm 0.13	74.95 \pm 1.74
250	5.63 \pm 0.45	90.73 \pm 0.51
125	11.29 \pm 0.97	98.14 \pm 0.28
62.5	20.78 \pm 0.36	100
31.25	28.47 \pm 0.51	100
15.6	35.61 \pm 0.83	100
7.8	42.85 \pm 0.79	100
3.9	50.34 \pm 1.42	100
2	62.96 \pm .64	100
1	75.13 \pm 0.35	100
0.5	82.97 \pm 0.11	100
0.25	89.56 \pm 0.28	100
0	100	100

Evaluation of cytotoxicity of meloxicam nanoemulsion in HCT-116 cancer cell line

The cytotoxicity of meloxicam nanoemulsion in comparison with meloxicam drug was compared. As shown in Table 1, meloxicam did not exhibit any cytotoxic action on the cells at all tested concentrations, and its IC_{50} value could not be determined. On the other hand, its inclusion in the nanoemulsion form resulted in significant decrease in the cellular viability, resulting in an IC_{50} value of 4.08 \pm 0.4 $\mu\text{g/ml}$. The non-cytotoxic effect of meloxicam as free form on the cells came in accordance with other authors [3], and they attributed this to their non expression of COX-2. This suggests the suitability of our prepared system in enhancing cellular uptake of drugs especially NSAIDs and allowing them to function as anticancer molecules in a non-COX dependant pathway such as apoptosis induction, as similarly encountered by other authors working on meloxicam [19]. The superiority of nanoemulsions could be related to their small size, which allow better cellular uptake, and hence enhanced anticancer activity [8]. Interestingly, when meloxicam was loaded in chitosan nanoparticles, an IC_{50} value could not be obtained for neither meloxicam nor the prepared nanoparticles on HT29 cancer cells [20], suggesting that the proper selection of nanocarrier is crucial to achieve good anticancer activity of the drug.

CONCLUSION

New indications are being discovered for both drugs and nanoparticles all the time. In the current work, it was proven that the nanoencapsulation of a drug as meloxicam would result in alteration of its mechanistic therapeutic effect. More futuristic studies are required to delineate the exact anticancer mechanisms of meloxicam in the nanoemulsion form.

Declaration of interest

The authors report no conflict of interest

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