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Assessment of hyaluronic acid-modified imatinib mesylate cubosomes through CD44 targeted drug delivery in NDEA-induced hepatic carcinoma

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ABSTRACT

This study aimed at the development of hyaluronic acid-functionalised imatinib mesylate cubosomes (HA-IM-CBs) that might be useful in CD44 targeting against hepatic cancer. The HA-IM-CBs had a 130.7 ± 2.92 nm particle size, −31.40 ± 2.76 mV zeta potential, and 76.14 ± 2.69% release. The architecture of HA-IM-CBs was confirmed using HR-TEM and AFM. When compared to plain IM and IM-CBs, *in vitro* experiments revealed that HA-IM-CBs outperformed by significantly reducing cell viability. DAPI staining and ROS corroborated the apoptotic effects. Biodistribution and Pharmacokinetics studies showed that HA-IM-CBs exhibit a higher drug concentration in tumour tissue and better pharmacokinetic activity. This is the first study to show that HA-IM-CBs had CD44 targeting activity against HCC. CD44 regulates apoptosis via Bcl-2 family proteins and caspases, which interact with HA. Higher levels of e-NOS, BAD, BAX, and Cyt C and lower expressions of Bcl-xl, i-NOS, and Bcl-2 demonstrated the anti-HCC potential of HA-IM-CBs in qrt-PCR investigations. The remarkable therapeutic potential of HA-IM-CBs began with substantial stimulation of CD44 regulated caspase-mediated mitochondrial apoptotic pathway, accountable for their anti-HCC activity. The perturbed metabolites are restored to acceptable levels as indicated by metabolomic studies (^{1}H NMR). Interestingly, the antineoplastic effect of HA-IM-CBs was proven to be potentially valuable against HCC.

1. Introduction

Hepatocellular carcinoma (HCC) has the sixth most widespread presence concerning cancers and is a major driver of cancer-related fatalities (Bray et al., 2018; Chen et al., 2019; Song et al., 2019). Every year, the rates of morbidity and mortality expand. Radial hepatectomy, liver transplantation, local ablation, and systemic pharmacotherapy are the prominent therapies for HCC (Boland and Wu, 2018). Large proportions of HCC patients are diagnosed at an advanced stage, necessitating non-surgical treatment or chemotherapy. Conventional

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; AFM, Atomic Force Microscopy; ANOVA, Analysis of Variance; BBD, Box-behnken design; CD44, cluster-determinant receptor; cDNA, Complementary deoxyrsibose nucleic acid; CAT, Catalase; CBs, cubosomes; DAPI, 4′,6-Diamidino-2- Phenylindole, Dihydrochloride; %DL, % Drug Loading; %EE, %Entrapment efficiency; EPR, Enhanced permeation and retention effect; FBS, Fetal Bovine Serum; FAs, Fatty acids; GMO, Glyceryl monooleate; GSH, Glutathione; GPC, Glycerophosphocholine; GI_{50}, Growth inhibition; HCC, Hepatocellular carcinoma; HA, hyaluronic acid; HPLC, High-performance liquid chromatography; HR-TEM, Hi-resolution Transmission Electron Microscopy; HA-IM-CBs, surface-modified hyaluronic acid and chitosan-coated CD44-targeted IM-CBs; HMDB, Human Metabolome Database; HDL, High density lipoprotein; IM, Imatinib mesylate; IM-CBs, Imatinib mesylate loaded cubosomes; ICH, International Conference on Harmonisation; LDH, Lactate dehydrogenase; LDL, Low density lipoprotein; MTT, 3-(4,5-Dimethylthiazol-2-yl)- 2,5-diphenyl tetrazolium bromide); NDEA, N-nitrosodiethylamine; NAG, N-acetyl-glycoproteins; NMR, Nuclear magnetic resonance; PS, Particle Size; PDI, Polydispersity Index; PBS, Phosphate-buffered saline; PC, Protein carbonyl; PCA, Principal component analysis; PLS-DA, Partial least square discriminant analysis; PUFAs, Polyunsaturated fatty acids; qRT-PCR, Quantitative Real-Time Polymerase Chain Reaction; RES, Reticulo-endothelial system; SOD, Superoxide dismutase; TBARS, Thiobarbituric acid reactive substances; TC, total cholesterol; TG, Triglycerides; VLDL, Very low-density lipoprotein; VIP, Variable influence on projection.

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