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Immunomodulatory activity of curcumin-entrapped poly D,L-lactic-co-glycolic acid nanoparticles in mice

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ABSTRACT

Background: Studies have shown that curcumin from *Curcuma longa* has a wide range of medicinal and immunomodulatory properties. These activities have, however, been hindered by its low bioavailability. Meanwhile, incorporation of nanoparticles has been shown to increase bioavailability of certain drugs. This study was, therefore, conducted to comparatively evaluate the immunomodulatory activity of free and nanoparticulate curcumin in mice.

Methods: Animals were sensitized with sheep red blood cells (SRBCs) and thereafter free and nanoparticulate curcumin were administered orally at doses of 5 mg/kg/day and 10 mg/kg/day for 10 days to healthy albino mice. The assessment of the immunomodulatory activity was carried out by determining the humoral and cell-mediated immune responses using haemaglutination and delayed-type hypersensitivity assays, respectively. Haematological components and some lymphoid organs of treated animals were further evaluated.

Results: The study showed that nanoparticulate curcumin stimulated higher early cell-mediated immune response at 5 mg/kg and 10 mg/kg when compared to control. While nanoparticulate curcumin significantly stimulated primary humoral immune response with 9.00 ± 1.00 antibody titre ($p < 0.05$), the free curcumin suppressed the immunity with 3.33 ± 0.67 antibody titre when compared to control. Similar result was observed with secondary humoral antibody titres. Production of white blood cells and weight of the lymphoid organs were also enhanced in the groups that received 10 mg/kg nanocurcumin.

Conclusion: This work showed that poly D,L-lactic-co-glycolic acid entrapped curcumin nanoparticle could increase bioavailability of curcumin for improved immunity.

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1. Introduction

The protection offered to the body system against specific diseases and infectious agents is achieved by the immune system.[1] The ability of the immune system to elicit this function is closely associated with certain compounds called immune modulators, which exert significant biological and pharmacological effects on the immune cells. Immunomodulators become very useful in abating the pathological impacts of immune-alteration associated diseases such as asthma, cancer, parasitic diseases, arthritis and ulcerative colitis. Many synthetic immunomodulators have been described, but plant-derived immune modulators are still in a developing stage. The need for more of the latter becomes paramount owning to severe side effects the synthetic immunomodulators pose on the immune system. For example, cyclophosphamide, an immunosuppressant, causes myelosuppression, nephrotoxicity, neurotoxicity, induction of diabetes, hepatotoxicity and induction of hypertension.[2] Medicinal plants are a rich source of substances that are known to induce paraimmunity, the non-specific immunomodulation of granulocytes, natural killer cells, macrophages and complement functions.[3] Plants such as *Punica granatum*, *Moringa oleifera*, *Phyllantus niruri*, *Cissus quandrangularis*, *Habenaria intermedia* and *Gymnema sylvestre* have been explored for their immunostimulatory properties.[4–7]

Turmeric (from *Curcuma longa*) is a mixture of curcumin [i.e., diferuloylmethane or 1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione)], demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin.[8] Curcumin has long been in use as a traditional remedy for diverse ailments, but its medicinal value was first documented in 1937 when used against biliary disease.[9] Since then, its therapeutic potential has been widely explored. It has been reported to have anti-bacterial, anti-fungal, anti-viral, anti-oxidative, anti-inflammatory and anti-proliferative activities.[10–12] The anti-cancer properties of curcumin against tumour cells have also been explored, and prevention of tumour initiation, promotion, metastasis and angiogenesis are the suggested mechanisms.[13,14] Curcumin has pleiotropic properties that modulate numerous targets including proteins (thioredoxin reductase, cyclooxygenase 2 (COX-2), protein kinase C (PKC), 5-lipoxygenase and tubulin), transcription factors, growth factors and their receptors, cytokines, enzymes and gene-regulating cell proliferation and apoptosis.[15–17] This multi-targeted behaviour made it to be able to perform a wide spectrum of actions, while smart drugs or therapeutic drugs have only one target and are eliminated from the cells if they do not reach the right compartment.[12] There have also been several clinical trials evaluating the possible anti-disease effect of curcumin in humans, as registered with the US National Institutes of Health, including studies on cancer, gastrointestinal diseases, cognitive disorders and psychiatric conditions.[18]

A large number of *in vitro* and animal studies have been conducted to evaluate the effect of curcumin on the immune system, and its adjunctive therapeutic potential for cerebral malaria management has been proposed.[19] It has been found to act at various different levels of the arachidonic acid immunomodulatory cascade and through effects on various enzymes and cytokines.[15,20] Although curcumin has enjoyed a wide range of applications, its clinical development has not been fully harnessed due to its quick metabolism and hydrophobicity.[21] Delivery systems such as liposome, biodegradable polymeric materials and chitosan are employed to increase the solubility and bioavailability of curcumin for improved biological actions.[22,23] The polymeric biodegradable nanomaterials have been especially favoured for the formulation of nanomedicines due to their grand bioavailability and controlled release.[24] The aim of this study, therefore, was to improve the immunomodulatory properties of curcumin through encapsulation with poly D,L-lactic-co-glycolic acid nanomaterial.

2. Materials and methods

2.1. Chemicals/drugs

Curcumin (from *C. longa* Linn), polyvinyl alcohol (PVA) (MW = 30–70 kDa) and D-mannitol were purchased from Sigma–Aldrich Ltd (St. Louis, MO, USA). Poly D,L-lactic-co-glycolic acid (PLGA) (intrinsic viscosity $\eta = 0.41$ dL/g, copolymer ratio 50:50, 45 kDa) was purchased from Purac Biochem, Holland. Dichloromethane and acetone were procured from Merck Serono Ltd. Water purified by Milli-Qplus system from Millipore (MQ water) was used.

2.2. Preparation of nanoparticulate curcumin

Curcumin-loaded PLGA nanoparticle (size: 291.2 ± 82.1 nm) was formulated by the method described by Busari et al.[25] Briefly, 5 mg of curcumin was added to the organic phase containing 50 mg of polymer dissolved in 3.5 mL of dichloromethane and 0.5 mL of acetone to constitute 1:10 (drug-to-polymer) ratio. The emulsion was continuously stirred at 300 rpm for 6 hours to evaporate the solvent, leaving behind the colloidal suspension of the drug-encapsulated nanoparticle in aqueous phase. The formulation was centrifuged at 16,000 rpm for 15 minutes and then washed three times. Dry powder was obtained by lyophilization of frozen sample in the presence of 5% mannitol as cryoprotectant.

2.3. Experimental animals

Swiss Albino male mice weighing between 20 g and 25 g were procured from colonies maintained at the Department of Pharmacology, University of Ibadan, Nigeria. All animal experiments complied with the International Guiding Principles for Biomedical Research involving Animals (CIOMS and ICLAS, 2012). The protocol was approved by the Animal Care Use and Research Ethics Committee (ACUREC) of the University of Ibadan, Nigeria. The animals were housed in clean cages and fed with standard pellet diet, with water *ad libitum*. The cages were maintained under standard environmental conditions in the Animal House at the Department of Zoology, University of Ibadan. Animals were weighed before and after the experiments.

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