Whole-cell Currents Recording from Ion Channels in Human Lymphocytes Treated with Anti-inflammatory Drugs in Nanoparticles Forms

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Abstract

Lymphocytes express an abundance of ion channels that are critical for their development and function. Many ion channels contribute to T cell-mediated autoimmune and/or inflammatory responses, so they are attractive targets for pharmacological immune modulations. In this study, we conduct patch clamp experiments to exam the whole cell currents from lymphocytes after nanoparticles exposure with the aim to test if nanoparticles exposure brings any electrophysiological changes for lymphocytes, and to compare the electrophysiological responses of lymphocytes to drugs in nanoparticles forms. Our result suggests a potential inhibition of effects of IBU N on lymphocytes. Such cytotoxicity of nanoparticles in Lymphocytes may be mainly associated with the early membrane damage. These results are also mirrored by the DNA damages occurred on lymphocytes after exposure of nanoparticles. Further detailed investigation is needed to explain the changes of Lymphocytes in response to NPs in real time and dose differences. This would provide useful information in the evaluation of toxicology of nanoparticles and in understanding the underlying mechanism of their effects on ion channels in health and diseases.

Keywords: ion channel, Nanoparticles, Lymphocytes, Ibuprofen (IBU)

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1. Introduction

Nanoparticles have potential toxic effects on human health since they can pass through biological membranes [1]. Ion channels are transmembrane proteins that mediate passive transport of ions, and the channels underlie a broad range of the most basic biological process, from excitation and signaling to secretion and absorption [2]. Ion channels in cell membrane are also targets for many toxins and drugs [3]. Studies of ion channels therefore can provide useful and informative clues for understanding the biophysics and pharmacology of these important and ubiquitous membrane proteins. However, a little knowledge about the effects of nanoparticles on ion channels is known. Therefore, it is necessary to examine the possible effects on ion channels after exposure to nanoparticles.

1.1 Ion Channel and Electrophysiology

Ion channels are pore-forming membrane proteins present in the membranes of all cells. Ion channels are key components in a wide variety of biological processes including establishing a resting membrane potential, shaping action potentials and other electrical signals by gating the flow of ions across the cell membrane, controlling the flow of ions across cells and regulating cell volume [2]. There are also a number of genetic disorders which disrupt normal functioning of ion channels and have disastrous consequences for the organism [4].

Therefore ion channels are a frequent target for searching new drugs and toxins [3].

Study of ion channels often includes biophysics, electrophysiology and pharmacology, utilizing various techniques including voltage clamp, patch clamp, immunohistochemistry, biochemistry, gene sequence comparison and mutagenesis, molecular and mathematical modelling and simulations, etc.

The fundamental properties of currents mediated by ion channels were analyzed by the British biophysicists Alan Hodgkin and Andrew Huxley as part of their Nobel Prize-winning research on the action potential, published in 1952 [5]. They built on the work of other physiologists, such as Cole and Baker's research into voltage-gated membrane pores from 1941 [6]. The existence of ion channels was confirmed in the 1970s by Bernard Katz and Ricardo Miledi using noise analysis [7].

It was then shown more directly with an electrical recording technique known as the "patch clamp", which led to a Nobel Prize to Erwin Neher and Bert Sakmann, the technique's inventors [8]. Hundreds if not thousands of researchers continue to pursue a more detailed understanding of how these proteins work. In recent years the development of automated patch clamp devices helped to increase significantly the throughput in ion channel screening. Patch clamping and its relevant technique are still a “golden” method in studying ion channel functions. It has four configurations as showen below (Fig1).

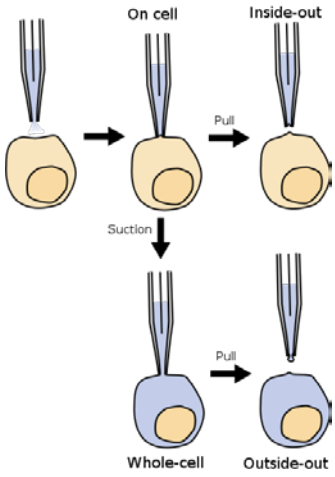

Figure 1: Four configurations.

(Ref: http://en.wikipedia.org/wiki/Patch_clamp)

There are some other variations combining with other techniques such as scanning probe microscopy to provide new ability to study electrophysiological changes with 3D cell membrane structures on nanometer scale and in real time [9], seen in the following discussion.

The determination of their molecular structure by Roderick MacKinnon using X-ray crystallography won a share of the 2003 Nobel Prize in Chemistry [9]. Because of their small size and the difficulty of crystallizing integral membrane proteins for X-ray analysis, it is only very recent that scientists have been able to directly examine what channels "look like." Most of what researchers have deduced about channel operation so far is established through electrophysiology, biochemistry, gene sequence comparison and mutagenesis.

1.2 Nanoparticles Interacting with Cell Membrane

Nanotechnology produces engineered nanomaterials (ENM) with new or enhanced physico-chemical properties in comparison to their micron-sized counterparts. Some of these properties, like the high surface area to volume ratio, make them potentially dangerous to humans as shown by many researches [11]. Nanotoxicology has been widely studied in all sorts of circumstance. Better understanding of the relationship between the ENM structure and the biological activities, especially how ENM interacts with cell membrane is needed to be able to promote the development of a new generation of ENM.

Increasing evidence linking nanoparticles (NPs) with different cellular outcomes necessitates an urgent need for the better understanding of cellular signaling pathways triggered by NPs. Oxidative stress has largely been reported to be implicated in NP-induced toxicity [12]. It could activate a wide variety of cellular events such as cell cycle arrest, apoptosis, inflammation and induction of antioxidant enzymes. These responses occur after the activation of different cellular pathways. The ability of NPs to interact with these signaling pathways could partially explain their cytotoxicity. The induction of apoptosis is also closely related to the modulation of signaling pathways induced by NPs. Newly emerged scientific areas of research are the studies on interactions between NPs and biological molecules in body fluids, cellular microenvironment, intracellular components or secreted cellular proteins such as cytokines, growth factors and enzymes and use of engineered NPs to target various signal transduction pathways in cancer therapy [13].

Nanoparticles exposure caused a variety of impairments to neuron, microglia in animals and aggravated the brain pathology, etc. Understanding the mechanism of toxicity of nanomaterials remains a challenge with respect to both mechanisms involved and product regulation. The properties of ion channels serves as a subtle indicator of the condition and viability of the cells. However, few studies directly address the interaction of NPs with ion channels.

Apart from direct experimental study about the interaction of NPs with cell membrane, Quantitative Nanostructure-Toxicity Relationships (QNTR) computational modelling technique is an effective alternative to experimental testing since it enables the prediction of (eco)-toxicological effects based on ENM structure only. The construction of QNTR model requires the integration of expertise of nanomaterial scientists, (eco)-toxicologists, and modelers from academia, regulatory agencies and industry (see more on discussion).

1.3 Lymphocytes Ion Channels with Nanoparticle Exposure

Lymphocytes express an abundance of ion channels that are critical for their development and function. Many ion channels contribute to T cell-mediated autoimmune and/or inflammatory responses and therefore are attractive targets for pharmacological immune modulation [14]. Lymphocytes are also suitable surrogate cells for cancers [15] and other diseases states [16] where inflammation is associated with increasing diseases incidence. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen are normally used for the treatment of anti-tumour effect in cancers. However this kind of pre-clinical and clinical studies can be lengthy and expensive. We recently chose to

exam the DNA damage in the Comet and Micronucleus Assays in peripheral blood lymphocytes of patients with respiratory diseases and healthy individuals using the nanoparticle forms of NSAIDs, aspirin and ibuprofen compared with their bulk forms [17].

In this study, we conduct patch clamp experiments to exam the whole cell currents from lymphocytes after nanoparticles exposure with the aim to test any electrophysiological changes. Responses of lymphocytes of patients with respiratory diseases and healthy individuals after exposure to nanoparticles and bulks forms of aspirin and ibuprofen were also compared with their DNA damages.

2. Materials and Methods

2.1 Ethical Approval

This study was approved by Leeds (Central) Research Ethics Committee (REC reference number: 09/H1313/37) and the Research Support & Governance Office, Bradford Teaching Hospitals NHS Foundation (ReDA number: 1202). Ethical permission was also provided by University of Bradford Research Ethics Sub-Committee on Research in Human Subjects (reference number: 0405/8).

2.2 Whole Blood Sample Collection

The patients were from the Outpatient Respiratory Disease Clinic of Dr. Badie K. Jacobs (St Luke's Hospital, Bradford, UK) and healthy control individuals from the University of Bradford. The criteria for patient selection included lung cancer (without prior chemotherapy or radiotherapy), chronic obstructive pulmonary disease (COPD) and asthma. Exclusion criteria included anaemia, other diseases beside their respiratory condition or previous occupational exposure to other NPs (e.g. silica or asbestos).

COPD patients were over 40 years of age with a 10 or more packs daily history of smoking and a fixed spirometric ratio of FEV1 to forced vital capacity (FVC) of <0.7 or radiological evidence of emphysema.

Asthma patients were diagnosed by their medical practitioner. These patients had symptoms of intermittent breathlessness, cough and wheeze. They had less than 10 packs daily history of smoking or non-smokers. They had variable and reversible airflow obstruction on spirometry. They also may have had atopy such as allergic rhinitis or hay fever or high IgE with eosinophilia

All individuals were given an information sheet, completed a questionnaire through interview and signed a consent form prior to approximately 10 ml peripheral blood being taken.

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2.3 Non-steroidal Anti-inflammatory Drugs

Ibuprofen USP was purchased from Albermarle Europe sprl, (Belgium). Pharmcoat 606 (HPMC) was kindly donated by Shinetsu (Japan). Aspirin and sodium lauryl sulphate (SLS) were purchased from Sigma–Aldrich, UK. Kollidon 30 (PVP K-30) was purchased from BASF (UK). Bulk and nano c suspensions ompounds of aspirin and ibuprofen were kindly prepared by Lena nanoceutics (Bradford, UK).

2.4 Nanomaterial Preparations

Suspensions of aspirin and ibuprofen with solid loads of 3% and 4% (w/w) respectively were prepared. The suspending medium consisted of: HPMC (0.5%, w/w), polyvinylpyrrolidone K-30 (0.5%, w/w) and sodium lauryl sulphate (0.1%, w/w) in deionized water [18]. The milling was carried out using Lena nanoceutics technology DM-100 machine [19].

250 ml of each suspension were milled using 150 ml of 0.2 mm yttrium stabilized zirconium beads (Glen mills, USA). The materials were recycled for 60 min in the milling machine before being discharged in an opaque glass bottle and stored in the refrigerator at (4°) for the duration of the experiments.

2.5 Particle Size Analysis

The particle size distribution of aspirin and ibuprofen nano-suspensions were determined using the dynamic light scattering technique of the Zetasizer Nano ZS (Malvern instruments, UK). Samples were measured at room temperature using disposable sizing cuvettes. All measurements were carried out in triplicate. The particle size of the stock suspensions were measured immediately after milling and then rechecked at the end of the experiments to ensure no significant change in particle size occurred during the various phases of the experiments.

2.6 Zeta Potential

The zeta potential for the suspensions was determined using Zetasizer Nano ZS (Malvern instruments, UK). The suspensions were diluted 1:100 using deionized water and measured at 25 C. Clear disposable zeta cells were used. Measurement duration was set as automatic with a minimum of 10 runs and a maximum of 100 runs. All measurements were made in triplicate.

2.7 Patch-Clamp Recording

An Axopatch 700B amplifier (Molecular Devices, United States) connected to a Digidata 1440A (Molecular Devices) was used to record whole-cell ion-channel currents [8, 20, 21]. All evoked currents were sampled at 5 kHz and filtered with 1 kHz low-pass Bessel filter. The extracellular solution contained (in mM) the following: NaCl, 125; KCl, 5; MgCl2, 1; CaCl2, 2.5; HEPES, 10; pH 7.4. The electrode internal solution contained (in mM) the following: KF, 120; MgCl2, 2; HEPES, 10; EGTA, 10; and CaCl2,1, pH 7.4. Both external and internal solutions were filtered with 0.1 μm sterile syringe filters (Pall Corporation). All experiments were carried out at room temperature.

2.8 Statistics

Statistical tests for the Comet assay showed that Gaussian normality was violated for many of the scale variables as endorsed by the Kolmogorov–Smirnov test. Therefore, non-parametric test procedures were adopted wherever necessary, such as the Kruskal–Wallis (K–W) and the Mann–Whitney (M–W) tests for independent samples. When testing intra-subject differences in DNA damage, the Wilcoxon Signed Rank test was applied [16].

Statistical analysis for the micronucleus assay: This was carried out using the chi-square test (X2) in 1000 cells per group to determine significant differences with p value set at * p = < 0.05 as recommended by Fenech [22].

All treated samples in the four groups (healthy individuals, asthma, COPD and lung cancer) were compared to their untreated lymphocytes. Also bulk forms in each group were compared directly to their nano forms.

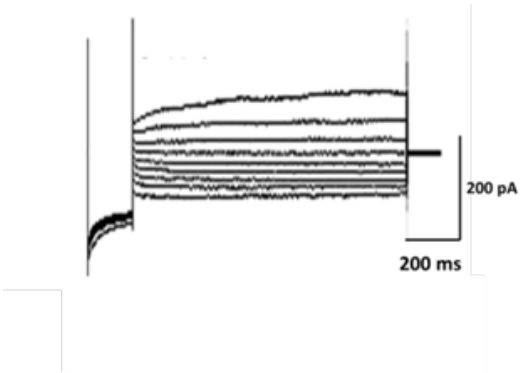

Figure 2: Representative recordings show effects of nanoparticles on the whole-cell currents by 500 µg/ml nanoparticles

3. Results

With the patch-clamp technique, whole-cell currents recordings from drugs-treated Lymphocytes were performed. A representative whole-cell recording obtained from the treated Lymphocytes with nano forms of Ibuprofen was presented in (Figure 2). As compared with untreated cells, drugs in nanoparticles form-treated Lymphocytes had lower whole-cell currents. The current–voltage (I–V) relationships for these recordings are shown in (Figure 3). In the presence of nanoparticles, the amplitude of total current (pA/pF) was decreased at the test potentials between −50 and +0 mV. These whole-cell currents were consistently evoked by applying 10 mV steps of 1000 ms duration from a hold potential of −50 to +30 mV (Figure 4). The whole-cell patch-clamp recordings demonstrated that 30 min treatment with drugs in nanoparticles form could inhibit the activities of ion channels 20% in Lymphocytes compared to that in Bulks form. These data suggest the potential inhibition effects of drugs in nanoparticles form on Lymphocytes.

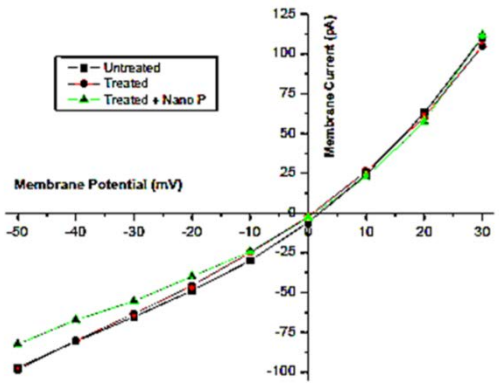

Figure 3: Voltage protocols used to obtain the current–voltage relationships

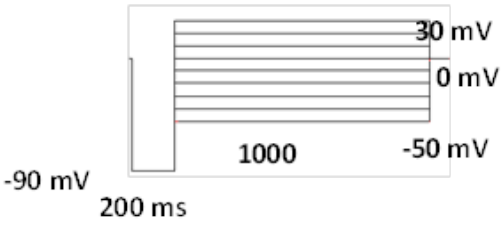

Figure 4: Current–voltage relationships correspond to 10 recordings obtained from untreated cells and nanoparticles-treated cells

Effects of nanoparticles on the whole cell currents of ion channels in Human Lymphocytes. Ibuprofen treatment in nanoparticle and bulk forms on ion channel activities of lymphocytes

DNA damage in peripheral lymphocytes from healthy individuals and respiratory disease patients treated ex vivo/ in vitro with aspirin and ibuprofen nanoparticles compared to their bulk forms are studied separately in [17]. Electrophysiology result above is mirrored by the DNA damages occurred on lymphocytes after exposure of nanoparticles [17]. All these findings fit well with the pre-clinical and clinical researches on the use of NSAIDs for the treatment of anti-tumour effects in cancers.

4. Discussion

Nanotechnology is providing science with a new platform in medicine which has the potential to provide disciplines such as diagnostics and clinical medicine, as well as basic research, with new materials in the nanometer range, that have many far reaching applications. Nanomaterials, such as nanoparticles, differ from other materials due to a number of special characteristics, including small particle size, large surface area, shape, chemical composition, and charge [11]. Together these characteristics give nanoparticles numerous advantages over other delivery systems, and the targeted delivery of drugs using nanocarriers for the treatment of diseases is a major focus of interest.

Even though there have been many advances in the area of bionanoscience, there is still very little known about the complex interaction of nanoparticles with the cell membrane, and the effect that this interaction can have on many diverse cellular processes. Nanoparticles at the cell membrane have the potential to interact with numerous cell signaling receptors, ion channels, transporters, and cytoskeleton machinery to control and regulate basic cellular and physiological processes.

Lymphocytes express an abundance of ion channels that are critical for their development and function. Many ion channels contribute to T cell-mediated autoimmune and/or inflammatory responses and therefore are attractive targets for pharmacological immune modulations.

The patch-clamp experiment here suggests a potential inhibition of effects of IBU N on lymphocytes. Although the involved intracellular biochemical mechanisms and ion channels in our observed nanoparticles toxicity remain to be determined, this study provides direct evidence that a dose of 500 μg/mL nanoparticle can cause membrane damage to Lymphocytes after 0.5h of exposure. Such cytotoxicity of nanoparticles in Lymphocytes may be mainly associated with the early membrane damage.

The further detailed investigation is needed to further explain the changes of Lymphocytes in response to NPs in real time and dose differences [23, 24]. These include how the nanoparticles interact with cells; in vitro & create test conditions that are more closely aligned with in vivo conditions; the extended electrophysiological recording method with Hopping probe scanning ion conductance microscope (HPSICM); nuclear magnetic resonance (NMR) spectroscopy. NPs form a protein corona when confronted with protein-containing solutions or full flood. Electrophysiological changes also need carefully interpreted.

Another approach worth to emphasize is quantitative structure-activity relationship (QSAR) modelling of nanomaterial toxicity, modelling the mechanisms of nanoparticle-lipid interactions and nanoparticle effects on cell membrane structure and function. The aim of this kind of study is to develop physically justified models and computational tools to quantitatively describe and understand the molecular mechanisms of nanoparticle-cell membrane interactions, which is considered to be a

crucial point in any predictive model of nanoparticle toxicity. It will address the mechanisms of nanoparticle protein corona formation, the protective function of the membrane, nanoparticle uptake into the cell, and the effect of nanoparticles on the cell membrane. It hopes to develop a consistent multiscale simulation scheme starting from nanoparticle-biomolecule interaction at the atomistic scale using molecular dynamics simulation, and then systematically constructing coarse-grained mesoscale models for simulating the structure and dynamics of the cell membrane perturbed by nanoparticles at the physiologically relevant time and length scales. It aims to eventually develop and test a universal method for evaluating the rates of nanoparticle translocation through membranes. Based on the information acquired from the simulations and analyzed together with available experimental data, the toxicological impact will be deduced. These approach will be applied to a range of common engineered nanoparticles, relating their physicochemical properties such as size and shape, surface charge, hydrophobicity and hydrophilicity, to the toxicological effects and develop a test suite allowing to make toxicity prediction on the basis of purely computational or limited vitro screening tests [25, 26].

Acknowledgments

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