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Original article

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Please cite this article as: Alhawmdeh M, Isreb M, Aziz A, *et al*. Interferon gamma liposome; a new delivery system to improve drug delivery in the treatment of lung cancer. *ERJ Open Res* 2021; in press (https://doi.org/10.1183/23120541.00555-2020).

Interferon gamma liposome; a new delivery system to improve drug delivery in the treatment of lung cancer

Maysa Alhawmdeh[1], Mohammad Isreb[2], Abid Aziz[3], Badie K. Jacob [3], Diana Anderson[1], Mojgan Najafzadeh[1*]

[1]Division of Medical Sciences, Faculty of Life Sciences, University of Bradford, Richmond Road, Bradford, West Yorkshire, BD7 1DP, UK.

[2] School of Pharmacy, Faculty of Life Sciences, University of Bradford, Richmond Road, Bradford, West Yorkshire, BD7 1DP

[3] Bradford Royal Infirmary, Bradford Teaching Hospitals NHS Foundation Trust, Duckworth Lane, Bradford, West Yorkshire, BD9 6RJ, UK.

To whom correspondence should be addressed:

*Dr Mojgan Najafzadeh:
School of Chemistry and Bioscience
Faculty of Life Sciences
University of Bradford
Richmond Road, West Yorkshire,
BD7 1DP, UK
. E-mails: m.najafzadeh1@bradford.ac.uk, d.anderson1@bradford.ac.uk

Declaration of interest

The corresponding authors confirmed that the authors have no conflicts of interest with this manuscript.

Keywords: lung cancer, interferon gamma, DNA damage, liposome, Comet assay Micronuclei.

Abstract

Lung cancer is one of the main causes of death worldwide. Published data show the use of interferons (IFNs) in treating lung tumours. Also, IFNs present a potential for antiproliferative, anti-angiogenic, immunoregulatory, and proapoptotic effects. The IFN-γ functions as an anticancer agent against various forms of cancer. This study aimed to investigate the effect of IFN-γ liposome (nano) on peripheral lymphocyte from 20 individuals in each group; lung cancer patients, compared to healthy individuals. The effectiveness of IFN- γ liposome against oxidative stress was also evaluated in this study. A concentration of 100U/ml of IFN-γ liposome was used to treat the lymphocytes in the Comet and micronucleus assays based on the preliminary test for the optimal dose. The lymphocytes from lung cancer patients presented with higher DNA damage levels than those of healthy individuals. In healthy individuals, IFN-γ liposome didn't cause to induce any DNA damage in the lymphocytes. Also, it caused a significant reduction in DNA damage in the lymphocytes from lung cancer patients in both the Comet and micronucleus assays. The 100U/ml of IFN-γ liposome significantly reduced the oxidative stress caused by H_2O_2 and appeared to be effective in both groups using the Comet and micronucleus assays. Results from both Comet and micronucleus assays were consistent. The data obtained indicated that IFN-γ in both forms (INF-γ bulk and INF-γ nano-liposome) may potentially be effective for the treatment of lung cancer and showed the ability of IFN-γ liposome to reduce the DNA damage more than the bulk form.

Introduction

The term lung cancer encompasses epithelial cancers in the mucosa of the bronchi and sometimes in the parenchyma of the lung, which exists in the trachea (windpipe), bronchi (airways) or lung air sacs (alveoli) (Travis, 2002, Lumb, 2016). Lung cancer accounts for roughly thirteen percent of the annual cancer cases worldwide. It is also the second most common type of cancer in males and females within the United Kingdom. However, its incidence is greater in males than females (Ferlay et al., 2015). In the UK, lung cancer is the leading reason for cancer death, with approximately 35 000 deaths a year. The diagnostic peaks for lung cancer are between the ages of 73 and 84 (Chivima, 2015), and the overall 5-year survival rate is lower than 10%, largely due to the majority of patients presenting with a late-phase of the disease, a point when treatment has little impact on survival (Ali et al., 2015). This cancer could be developed or extended to the airways resulting in symptoms, such as cough, airway obstruction, and haemoptysis. The tumour can metastasise to the area of the thorax causes compression. It might also invade the chest wall and develop more extention via the hilar, mediastinal, and supraclavicular nodes (Mitchell and Kennedy, 2014). Furthermore, it enhances transformations in the central or peripheral nervous system, leading to anorexia and causing a disturbance in hormone production. Cancer metastasises via the blood to different parts of the body, specifically to the liver, brain, adrenal gland and axial skeleton (American Cancer Society, 2015).

Studies have shown that cigarette smoking is the main risk factor in lung cancer patients. Current smokers have a 15-fold increase in the risk of death from lung cancer compared with lifelong non-smokers (Doll et al., 2005). On the other hand, this risk decreases significantly in individuals who cease smoking before middle age (Peto, 2000). The risk of lung cancer is also elevated following exposure to Environmental Tobacco Smoke (ETS). An estimated 14% to 15% of lung cancers are caused by exposure to ETS (Parkin, 2011). Eighty-five percent of lung cancer patients are non-small cell lung cancer (NSCLC), including adenocarcinoma, Adenosquamous cell carcinoma, squamous cell carcinoma, and large cell carcinoma.

IFN-γ is produced mainly by Th1 CD4+ T cells, CD8+ T cells and natural killer (NK) cells. Additionally, recent studies have indicated that other cells such as B cells, NKT cells and professional antigen-presenting cells (APCs) may also be a source of IFN-γ. This secretion is controlled primarily by IL-12 and IL-18 (Mojic et al., 2017). IFN-γ plays a major role as antiviral, immunoregulatory and anti-tumour, through interaction with specific cell-surface receptors, including four transmembrane polypeptides receptor complexes: two ligand-binding chains (IFN-γR1) and two signal-transducing chains (IFN-γR2), which are required for signal transduction (Castro et al., 2018). The most critical transduction pathway stimulated by IFN-γ is the Janus kinase (Jak)–signal transducer and activator of transcription 1 (STAT1) pathway. This contributes to the antiproliferative effects of IFN-γ on different cell types, as it has been elucidated that both pathways have the potential to inhibit tumour progression and kill pathogen-infected cells (Seif et al., 2017). This is done by binding two IFN-γ R2 subunits with two IFN-γ-bound IFN-γ R1 chains (Melmed et al., 2015). After the receptor complex association is induced, Jak1 and Jak2 activate and phosphorylate Tyr440 of IFN-γ R1, forming a docking site for STAT1. STAT1 is activated by phosphorylation at Tyr701, leading to homodimerisation of STAT1 and nuclear translocation. The STAT1 dimers interact with specific DNA sequences called IFN-γ-activated sequences (GAS), present in the promoter regions of IFN stimulated genes, and responsible for regulating their transcription (Krause et al., 2006, Kulling et al., 2018). The entirety of these mechanisms are presented in Figure 1 (Zaidi and Merlino, 2011).

The transcriptional activity of STAT1 is stimulated through kinases. These kinases are mitogen-activated protein kinase (MAPK), protein kinase C(PKC), and phosphoinositide 3-kinase (PI3K)/AKT, which phosphorylates STAT1 in the transactivation domain (Zaidi and Merlino, 2011). The mechanism of (STAT1) pathway plays a positive role by regulating the expression of genes encoding several antiproliferative and proapoptotic molecules, such as IFN regulatory factor 1 (IRF-1). However, if IRF-1 is inhibited, the INF-γ activates the proliferative signals. On the other hand, when the IRF-1 is elevated, the INF-γ stimulates apoptotic signals (Bernabei et al., 2001, Rettino and Clarke, 2013). In mice, those with deficient IFN-γ spontaneously develop lung epithelial malignancies and lymphoma. Furthermore, mice with a deficient IFN-γ receptor and STAT1 manifest tumour

growth following chemical carcinogen treatment, which emphasises the ability of IFN-γ to act as an anticancer agent (Gao et al., 2018). Furthermore, preliminary research indicates that IFN-γ, given as a single agent, has a measurable effect as an antitumor agent in advanced NSCLC (Shen et al., 2018).

Liposomes are artificial vesicles made from single or multiple phospholipid layers (lamellas) with spherical, self-assembled shapes. Their sizes typically range from tens of nanometres up to hundreds of micrometres depending on their preparation methods (Mishra et al. 2011; Laouini et al. 2012). They can also be made from non-toxic surfactants, cholesterols, and membrane proteins (Alavi et al. 2017). Liposomes have been used extensively as drug delivery carriers and utilised for biomedical and biotechnological purposes (Joshi et al. 2016). The encapsulation of the drug into liposomes can reduce the toxicity, enhance the pharmacokinetics of the drug and result in more efficient therapeutic effect (Shi et al. 2015).

Moreover, liposomes can increase bioavailability and improve the solubility of poorly water-soluble drugs (Ali et al. 2013). A recent study adopted the IFN-γ liposome-based drug delivery method by modifying it with cyclic peptides in order to initiate and anti-fibrotic effect for the therapy of hepatic fibrosis on the rat. It was found that the sterically modified IFN-γ liposomes have a greater anti-fibrotic effect of activating the hepatic stellate cells by 7.24-fold and 2.95-fold compared to free IFN-γ with less adverse side effects (Li et al., 2012a).

The research primary objective was to study the DNA protective effect of interferon-gamma bulk and interferon-gamma liposome on lymphocytes from lung cancer patients compared to healthy individuals.

In this study, the effect of interferon-gamma and interferon-gamma liposome on DNA damage was detected in peripheral blood lymphocytes of lung cancer patients and healthy individuals by using the Comet micronucleus assays. The Comet assay was used in this study due to its sensitivity and simplicity for analysis of the genotoxicity of DNA in lymphocytes as presented in previous studies (Tice et al., 2000, Anderson et al., 1997, Singh et al., 1988).

Materials and methods

Materials

Interferon-γ (≥98% purity; Cat No. 17001), Mitomycin C (Cat No. M0503) and fetal bovine serum (FBS) (Cat No. F7524) were purchased from Sigma-Aldrich (Gillingham, UK). RPMI 1640 medium (Cat No. R8758), phytohaemagglutinin (PHA) (Cat No. L1668) and penicillin-streptomycin solution (Cat No. P4333) were also obtained from Sigma-Aldrich. All other chemicals used in the different tests were from Sigma-Aldrich Company Ltd. (Sigma Chemical Ltd., Gillingham, UK). Prior to using the Interferon-γ, the lyophilised powder was reconstituted in double distilled water to form the stock solution. It was then diluted in RPMI medium with 10% foetal calf serum and stored at -20 ºC.

The dose-response experiments were carried out to determine the optimal doses of IFN-γ bulk and liposome and H_2O_2 used throughout the study. A fixed-dose of both forms 100U/ml of IFN-γ bulk and 100U/ml of IFN-γ liposome were used as treatment. The classic genotoxic compound hydrogen peroxide (H_2O_2) (75 μM) was used as a positive control (PC).

Preparation and Characterisation of Liposomes

Liposomes were prepared using the thin film rehydration method. 1,2-Dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DPPG),1,2-dioleoyl-sn-glycero-3-phsphoethanolamine (DOPE) and cholesterol were dissolved in Dichloromethane (DCM) and methanol (3:1 v/v). The solution was then transferred to a rotary evaporator with a 25ml round bottom flask. The organic solvent mixture was evaporated at 40^{o}C under vacuum for 6 hours, to ensure no traces of the organic solvents were left. The thin film was then hydrated by 10 ml of PBS solution of IFN-γ for 1 h at 60^{o}C using bath sonicator (150 W). The sample was then subjected to 4 repeated freeze-thaw cycles (freezing at -20C and thawing at 60C in the bath sonicator). Furthermore, the blank liposome was prepared from the same components without adding the IFN-γ.

Average size and polydispersity index (PDI) of the liposome preparations were determined by dynamic light scattering (DLS) using zetasizer ZS (Malvern Instruments, UK). All measurements were performed in triplicate.

Ethical Approval

Ethical approval was obtained to execute the Comet and micronucleus assays for the study of IFN-γ and IFN-γ liposome. This study received ethical approval from Leeds East Research Ethics Committee (REC number: 12/YH/0464), the University of Bradford Research Ethics Sub-Committee on Research in Human Subjects (Ref: 0405/8) and the Research Support and Governance office, Bradford Teaching Hospitals, NHS Foundation (Ref: RE DA 1202).

Sample preparation and Comet assay

After taking consent from healthy non-smoking volunteers and lung cancer patients, whole blood samples were collected by venepuncture in 9 ml lithium heparin-coated tubes and clearly labelled for ease of identification. Samples were diluted in a ratio of 1:1 with Roswell Park Memorial Institute (RPMI) 1640 Medium (RPMI-1640), and then mixed with 10% Dimethyl sulfoxide (DMSO). The diluted blood solution was divided and transferred to labelled 1.5 ml Eppendorf® tubes, which were closed tightly and stored at -80°C.

The Comet assay was prepared according to (OECD, 2016, Najafzadeh, 2016). In brief, 100 µl microliters of whole blood samples were incubated for 30 minutes at 37°C with different test agents, to make the final volume of 1000µl with RPMI 1640 media (with sodium bicarbonate, without L-glutamine, liquid, sterile-filtered, suitable for cell culture). The test agents included a positive control of 75µM H_2O_2, blank liposome IFN-γ 100U/ml and IFN-γ liposome in order to test the effect of IFN-γ in a highly oxidising environment; 100u/ml of IFN-γ and IFN-γ liposome were treated in the presence of 75Mm H_2O_2. An untreated sample was used as a negative control. The samples were then

incubated at 37°C for 30 mins. Following incubation, the samples were centrifuged at 3000 rpm for 5 minutes. The method used thereafter was followed as described in (Tice et al., 2000, Anderson et al., 1997, Singh et al., 1988).

The slides were coded before scoring; per slide, 100 nuclei were scored using a fluorescent microscope at 20 X magnification. This was in connection with a CCD camera using Komet 6 software and Kinetic Imaging (Andor Technology Ltd, Belfast). Olive tail moment and % Tail DNA were used simultaneously to decrease variability in the results (Najafzadeh et al., 2016).

The cytokinesis block micronucleus (CBMN) assay

Five hundred microliters of whole blood were added to a T25 cm^2 Corning culture flask containing 4.5 ml RPMI-1640 medium supplemented with 1% of Penicillin-streptomycin, 15% Foetal bovine serum and 25 mM HEPES and L-Glutamine with end concentrations of 15 and 1%, respectively, followed by 100 µl of phythaemagglutinin (PHA) (2.5%). In the next 24 h, 50 µl of blank liposome was added to the negative control. Two positive controls were used with 10 µl of mitomycin C (0.4 µM) used as positive control 1, and 50 µl of H_2O_2 served as positive control2. Fifty microliters of 100U/ml IFN-γ and IFN-γ liposome were added to their respective flasks and the final flask contained, IFN-γ liposome and H2O2. Fifty µl of cytochalasin-B (6 µg/ml, Sigma) was added to each flask and cultured at 37◦C in the presence of 5% CO_2 for 44h. The CBMN procedures were performed regarding to Fenech et al. (Fenech et al., 2016, Fenech, 2007) .

Numerous cytological scoring parameters were used to assess DNA damage. Micronuclei (MN) were scored from both binucleated (BiNC) and mononucleated cells (MonoNC). Nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) were only scored for BiNC (Fenech et al. 2011). The nuclear division index (NDI) was calculated using the equation of NDI = M1 + 2(M2) + 3(M3) / N, where M1 = mononucleated cells, M2= binucleated cells, M3 = multinucleated cells and N = total number of viable cells scored (Fenech et al. 2003).

Cell viability

The viability of cells was detected after 30 minutes of incubation of cells with different concentrations of treatments. Lymphocytes were isolated from whole blood using ficoll-paque as described in (Bausinger and Speit, 2016). Cell viability was then determined by the trypan blue staining method described in (Chan et al., 2020) and the Cell counting kit-8 (CCK-8) method (Riss et al., 2016). The concentrations with cell viability of ≥75% were used in all experiments (Henderson et al., 1998).

Statistical Analysis

The final data were expressed as mean values with standard errors, the results were analysed using one-Way analysis of variance (ANOVA) with Dunnett's multiple comparisons test using GraphPad prism 8.1.2. The p values were considered significant at $P<0.05$(*), $p<0.01$(**), $p<0.001$(***).

Results

The viability of lymphocytes from healthy individuals and lung cancer patients was assessed after treatment with chemicals used in this study was between (75 -85%) using trypan blue dye exclusion. The viability of lymphocytes from healthy individuals and lung cancer patients treated with different treatment groups was also confirmed using the CCK-8 assay ≥ 75 after 24 h treatment (Fig.1).

Comet assay: IFN-γ liposome vs Bulk

The data from the Comet assay results showed that lung cancer patient lymphocytes were more susceptible to DNA damage than healthy individuals, in % Tail DNA and OTM, when compared with groups that received no treatment (***$p < 0.001$) as presented in Table1, Figs. 2 and 3. A significant increase in % tail DNA and OTM in lymphocytes from healthy volunteers and lung cancer patients after exposure to PC (75 μM H2O2) (***$p \leq 0.001$) Figs. 2 and 3.

Lymphocytes obtained from 20 healthy individuals in figures 2 and 3 showed that 100U/ml IFN-γ B and IFN-γ liposome was clearly unaffected in lymphocyte cell when measuring % Tail DNA and OTM compared to untreated cells. In contrast, the H_2O_2-induced DNA damage was significantly reduced by 100U/ml IFN-γ B and IFN-γ liposome in lymphocyte from healthy individuals compared to the PC (75 μM H_2O_2) (***p ≤ 0.001). In contrast, IFN-γ liposome was more effective in reducing the DNA damage more than IFN-γ B as presented in table 1.

Lymphocytes from lung cancer patients (n=20) also showed a significant decrease in % tail DNA and OTM from 10.97% (% tail DNA) and 2.18 (OTM) compared to the untreated control groups to 8.42% (% tail DNA), and 1.38 (OTM) , respectively, when cells were treated with IFN-γ B whereas the reduction in % Tail DNA and OTM was more effective by using IFN-γ liposome which showed a significant decrease in % tail DNA 6.65% and 1.07 (OTM) . Furthermore, cells treated with IFN-γ B and IFN-γ liposome co-supplemented with 75 μM H_2O_2 showed significant decreases in OTM and % tail DNA compared to the PC (75 μM H2O2) (***$p \leq 0.001$).

CBMN: Effect of IFN-γ liposome

The lymphocytes from healthy individuals and lung cancer patients were treated with 75 μM H_2O_2, 0.4 μM mitomycin c (MMC),100U/ml of IFN-γ B, IFN-γ liposome and 100U/ml of IFN-γ bulk, IFN-γ liposome with 75 μM H_2O_2. They were then assessed for MNi induction using the CBMN assay, as presented in Figure 4. In contrast, lymphocytes from healthy individuals treated with 100U/ml of IFN-γ bulk and IFN-γ liposome, showed no effect in MNi frequency compared to untreated cells. The MMC and 75 μM H_2O_2 displayed a significant increase in the MNis of lymphocytes (***p<0.001), but 100U/ml of IFN-γB and IFN-γ liposome co-supplemented with H_2O_2 showed a significant decrease in MNis of lymphocytes *(*p<0.05)* as in Figure 4. It was evident that the 100U/ml of IFN-γ and IFN-γ liposome showed a significant reduction in the number of MNis in lung cancer patients, but the reduction by IFN-γ liposome was more (***p<0.001), compared to untreated cells. Furthermore, the MMC and 75 μM H_2O_2, showed a significant increase (***p<0.001) in the MNi number on lymphocytes compared to the untreated cells.

There are numerous cytological scoring parameters, including biomarkers of cell mitotic division such as mononucleated cells (MoNC), binucleated cells (BiNC), and multinucleated cells (MultiNC). From these parameters values, the NDI was calculated for healthy individuals and lung cancer patients, respectively. The mean values for the NDI for all treatment groups were within the normal range, as shown in Table 2. Table 2 also displays the chromosomal instability parameters in the form of the mean of NPBs, NBUDs per 1000 BiNC and MNi frequency in MoNC. The table clearly showed that the frequency of MNi in BiNC of untreated cells is higher than in lymphocytes treated with IFN-γ liposome on lung cancer patients.

Discussion:

This study investigated the efficacy of IFN-γ and IFN-γ liposome in human lymphocytes originating from groups of twenty healthy individuals and twenty lung cancer patients, using the Comet assay and 5 individuals in each group for the CBMN assay. Different *in vitro* and *in vivo* studies have shown the promising applications of IFN-γ in the field of health as a treatment displaying pleiotropic immunomodulatory, antiviral, antimicrobial, anti-neoplastic, pro-inflammatory activities (Castro et al., 2018). Furthermore, the potent inhibitory effects of IFN-γ in various tumour models, such as bladder carcinoma, colorectal cancer, ovarian cancer, and adult T cell leukaemia, human pancreatic carcinoma cells, and NSCLC have been evidenced(Mojic et al., 2017, Zaidi and Merlino, 2011).

Research has demonstrated that IFN-γ can induce apoptosis in human glioblastoma T98G and U87MG cells (Das et al., 2009), and inhibited the proliferation of four pancreatic cancer cell lines in vitro (Wu et al., 2016). Other studies indicated that IFN-γ was responsible for apoptosis in Ovarian Cancer Cells *in vivo* and *in vitro* (Razaghi et al., 2017). Contrarily, none of the previous studies has focused on the effect of IFN-γ on DNA damage in human lymphocyte from lung cancer patients through the use of the Comet and CBMN assay.

The obtained results showed that the treated lymphocytes from healthy individuals with IFN-γ B and IFN-γ liposome did not induce significant DNA damage when compared to the untreated cells. However, when the same concentration of IFN-γ in both forms was used on lung cancer patients' lymphocytes, DNA damage was reduced compared to the untreated cells. Still, the reduction of DNA damage that occurred by the liposome form of IFN-γ was greater than from the IFN-γ B (Figures 2,3). This could be possible due to the biocompatibility and enhanced cellular interaction of liposomes compared to their larger particles (Bozzuto and Molinari, 2015). The Comet assay results showed that IFN-γ B and the liposome form have a protective effect against H_2O_2, which induces damage in DNA due to oxidative stress in healthy individuals and lung cancer patients' lymphocytes. DNA damage significantly decreased compared to the PC. The effective results of IFN-γ liposome against H_2O_2-induced damage could be due to the ability of liposome in preventing cell membrane alterations rather than its direct interaction with H_2O_2 (Trif and Craciunescu, 2015).

The micronucleus assay was used to investigate the influence of IFN-γ B and liposome on the lymphocytes from healthy individuals and lung cancer patients at the chromosomal level. The CBMN assay has been a critical test in the detection of the genotoxicity of different compounds by measuring micronuclei and other chromosomal abnormalities such as NPBs, a biomarker of dicentric chromosomes, and by measuring NBUDs, which are a biomarker of gene amplification (Cho et al., 2020). MNis are cytoplasmic bodies generated as a consequence of disorder during the cell cycle division. Subsequently, when the centric fragments or whole chromosomes are incapable of travelling to opposite poles during anaphase, the nuclear envelope forms around the lagging chromosomes and fragments, which gradually take on the morphology of an interphase nucleus, which is smaller than the main nuclei in the cell (Fenech et al., 2011, Luzhna et al., 2013). In this assay, the dividing cell cytokinesis is inhibited with cytochalasin B (Cyto B) (Doherty et al., 2016).

In this study, amongst the different types of cells counted, specific consideration was made for the presence of MNi in the treatment groups since MNi are indicators of chromosomal breakage, loss, rearrangement, necrosis and apoptosis (Fenech, 2007). Furthermore, MNi in binucleated cells only

showed the damage after treatment, which decreased the probability of scoring the pre-existing damage (Li et al., 2012b). Mitomycin C (MMC) is recognised as an anti-tumour antibiotic, clastogenic, and genotoxic compound. Therefore, MMC served as a positive control in this study. MMC induced MNi production in binucleated cells more than mononucleated cells (Table 2). The percentage of BiNC after scoring 1000 cells was within the normal range in lymphocytes cultures for each individual. On the other hand, the percentage of mutliNC in cell culture was low, thus elucidating that cytochalasin B inhibited cell division after one cell cycle. Meanwhile, the NDI values were normal for all experiments (Table2).

From this assay, the frequency of MNi in the lymphocytes from healthy individuals and lung cancer patients decreased when treated with IFN-γ and IFN-γ liposome compared to the NC for each group. In contrast, the IFN-γ liposome showed a greater reduction in MNi frequency in lymphocyte. Hydrogen peroxide was used in this assay and functioned as expected, inducing the MNi production on the lymphocytes from both healthy individuals and lung cancer patients. The combination of IFN-γ B and IFN-γ liposome with H_2O_2 reduced the frequency of MNis compared to H_2O_2 in patients' lymphocytes. This study's most important finding was that IFN-γ liposome had exhibited higher DNA damage reduction effects at 100U/ml concentration than IFN-γ B.

The Comet assay data showed that IFNγ in bulk and liposome form reduced DNA damage in the lymphocytes from healthy individuals and lung cancer patients compared to the untreated cells (NC).

Our results were consistent with a previous study where IFN-γ induced early stimulation of PARP (the DNA repair enzyme) when the cells were exposed to proapoptotic agent (Saint Jean et al. 1999). Furthermore, DNA damage was decreased when the IFN-γ B and liposome were treated with H2O2 in the lymphocyte from healthy individuals and lung cancer patients compared to the PC. This denotes the ability of IFN-γ to protect against DNA damage in lymphocytes from oxidative stress. The CBMN assay followed a similar pattern to the Comet assay results. It also showed a reduction in MNi frequency in the lymphocytes, thus implying the potential facilitation of DNA repair.

In general, IFN-γ liposome (100IU/ml) has shown better protective effects than its larger particle counterpart, bulk at the same concentrations of 100IU/ml each. This could be possible due to the enhanced solubility of liposome-coated IFN-γ in an aqueous medium stabilising various therapeutic agents such as proteins and nucleotides. Their size, surface charge and composition are favourable conditions for their effective results (Moghimi and Agrawal 2005; Barenholz 2012; Cern et al. 2012) . Many hydrophilic and hydrophobic biomolecules can be encapsulated in liposomes and be protected from interactions with the external environment. Liposomes inhibit the metabolism of the drug before reaching the target cells plus protect the drug's interaction with healthy cells enhancing the therapeutic index of the drug. It has been well documented that the liposome form of any therapeutic drug shows enhanced pharmacokinetic properties and biodistribution than its free form. The morphology of liposomes is similar to that of the cellular membranes, making them an ideal drug-carrier system (Bozzuto and Molinari 2015).

In conclusion, the data obtained from the Comet and micronucleus assays indicated that IFN-γ in both forms might potentially be effective for lung cancer patients. Overall data from the present study proposes that IFN-γ could protect and defend against lung cancer through cell cycle arrest of cancer cells and repair mechanisms and liposome can potentially be used as an alternative better drug delivery system in various conditions.

Acknowledgement

The authors would like to thank the clinical staff of Bradford Royal Infirmary Hospital and St. Luke's Hospital for their assistance in this study. The Sponsorship by the Mutah university of a PhD studentship to Maysa Alhawamdeh is gratefully acknowledged. The Sponsor played no part in the conduct of the work or the writing of the manuscript.

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Table.1 The mean values of 20 experiments on blood samples from healthy individuals and lung cancer patients expressed as OTM and % DNA tail, including the statistical significance and SEM.

	Healthy individuals		Lung cancer patients	
	% DNA tail	Mean OTM ± SEM	% DNA tail	Mean OTM ± SEM
Untreated cells (NC)	8.26±0.32	1.37±0.05	10.97±0.52	2.18±0.15
PC-75 µM H_2O_2	15.61±0.83***	3.25±0.34***	15.69±0.71***	3.32±0.2***
Blank liposome	9.76±0.43 ns	1.57±0.12 ns	9.87±0.29 ns	1.71±0.08 ns
Naked IFN-γ 100U/ml	7.73±0.31 ns	1.26±0.06 ns	8.42±0.32**	1.38±0.07***
IFN-γ liposome 100U/ml	6.70±0.24 ns	1.046±0.05 ns	6.65±0.34***	1.07±0.07***
IFN-γ liposome+H_2O_2	8.53±0.36 ns	1.41±0.08 ns	8.61±0.38**	1.49±0.09**
Naked IFN-γ +H_2O_2	8.67±0.33 ns	1.45±0.06 ns	8.87±0.29*	1.54±0.06*

Table 2. The mean of different parameters for chromosomal damage in healthy individuals and lung cancer patients.

Subject	Treatment Group	Mean of NDI	Mean of % BiNC	Mean of % Multi	Mean per 1000 BiNC cells			Mean of % MNi in MoNC
					BiMNi	BiNPB	BiBuds	
Healthy volunteers	Untreated Lymphocytes	1.92+0.03	60.6+1.6	16.2+1.15	0.4+0.24	0	0	0
	75 μM H_2O_2	1.96+0.03	60.6+0.4	18.4+1.56	12.2+1.11	0.2+0.2	0	6.2+1.59
	0.4 μM MMC	1.88+ 0.06	59.2+2.4	15.6+3.81	18+2.09	0	0	8.4+1.8
	Naked IFN-γ	1.87+0.02	58.8+0.58	14.2+1.2	1.4+0.24	0	0	1.2+0.37
	Blank liposome	1.93+0.04	60.2+1.68	17+1.51	0.4+0.24	0	0	0
	IFN-γ liposome	1.98+0.03	61.6+0.4	19.4+1.4	1.2+0.2	0	0	0.6+0.4
	IFN-γ liposome+H_2O_2	1.96+0.02	59+2.28	19.8+2.72	4.2+0.86	0	0	3.2+1.28
	Naked IFN-γ +H_2O_2	1.92+0.07	59.6+1.91	17.6+4.45	4.8+0.96	0	0	4.4+1.43
Lung cancer patients	Untreated lymphocytes	1.90+0.03	58.4+2.2	16.4+1.43	7.4+0.6	0.2+0.2	0	3.4+0.4
	75 μM H_2O_2	1.89+0.03	56+2.46	17+2.07	17+1.3	0.6+0.4	0	6.4+1.2
	0.4 μM MMC	1.93+0.02	58+2.44	18+1.51	20.6+0.81	1.8+0.8	0.2+0.2	9+1.76
	Naked IFN-γ	1.97+0.03	59.6+0.5	19.2+1.59	3+0.31	0	0	3+0
	Blank liposome	1.91+0.02	60.8+0.86	15.2+1.42	5.6+0.4	0.2+0.2	0	3.2+0.48
	IFN-γ liposome	1.87+0.04	57.8+1.24	15.2+2.2	2.2+0.37	0	0	1.8+0.2
	IFN-γ liposome+H_2O_2	1.99+0.03	58.6+2.18	21+2.6	6.6+1.2	0	0	4+0.7
	Naked IFN-γ +H_2O_2	1.98+0.03	61+1.34	18.6+1.56	8.6+0.92	0	0	5+1.37

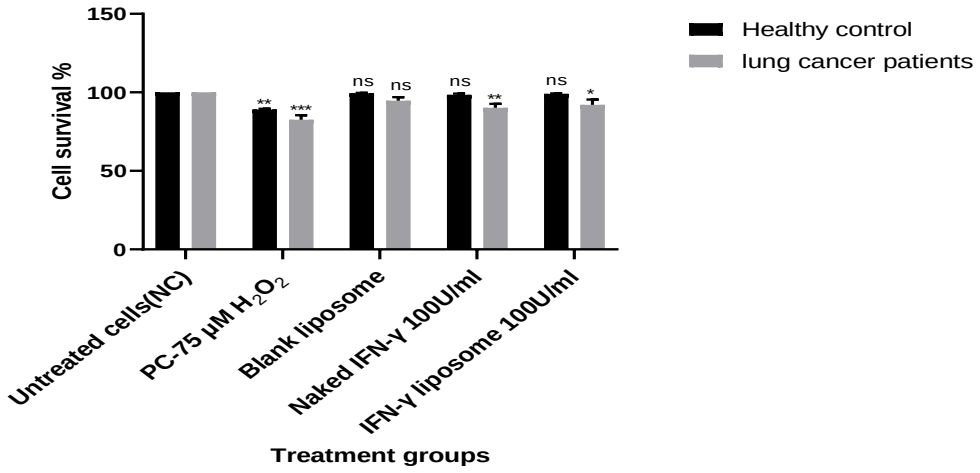

Fig. 1: % survival in 10×10^4 cells of various test treatments in lymphocytes from healthy individuals and lung cancer patients using CCK8 assay. Error bars show mean values ± SE, n = 3. *p<0.05; ** p<0.01; *** p<0.001, ns not significant

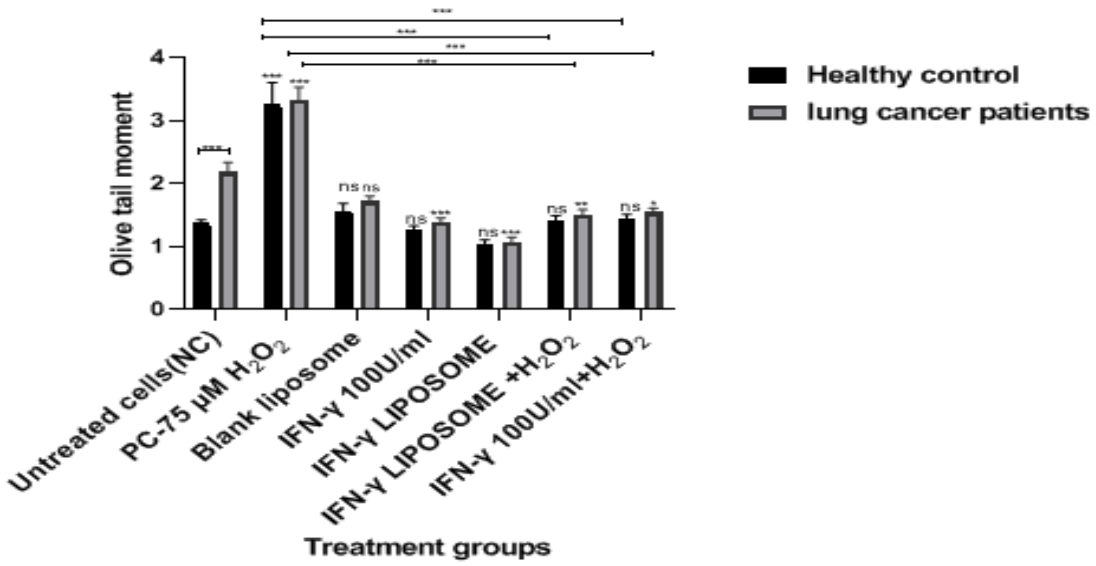

Fig. 2: DNA damage measured as mean OTM before and after treatment with bulk and liposome form of IFN-γ in human lymphocytes from healthy individuals and lung cancer patients in the Comet assay. n= 20 in each group.

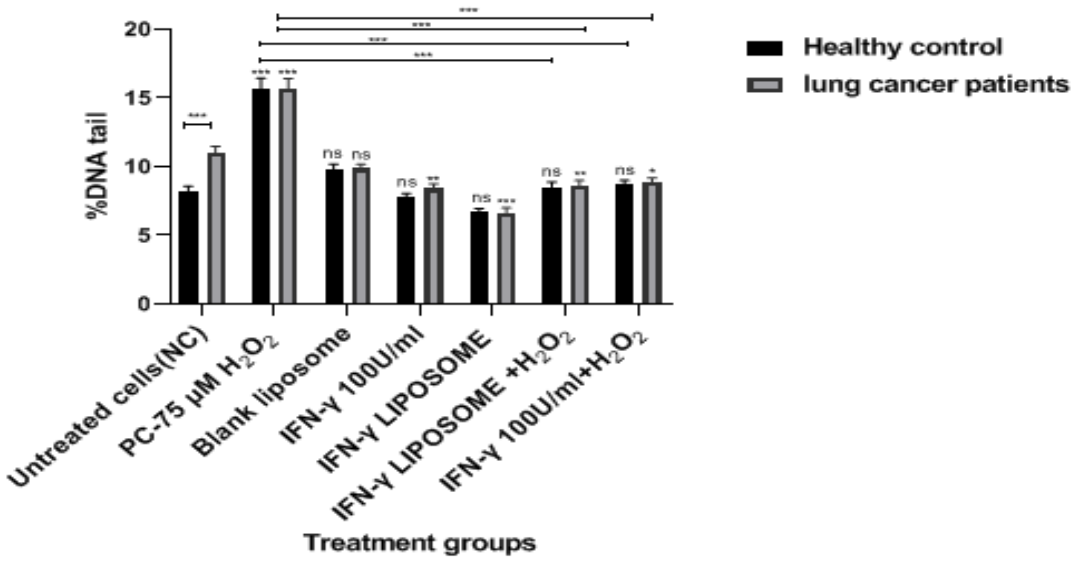

Fig. 3: DNA damage measured as mean % tail DNA before and after treatment with bulk and liposome form of IFN-γ in human lymphocytes from healthy individuals and lung cancer patients in the Comet assay. n=20 in each group.

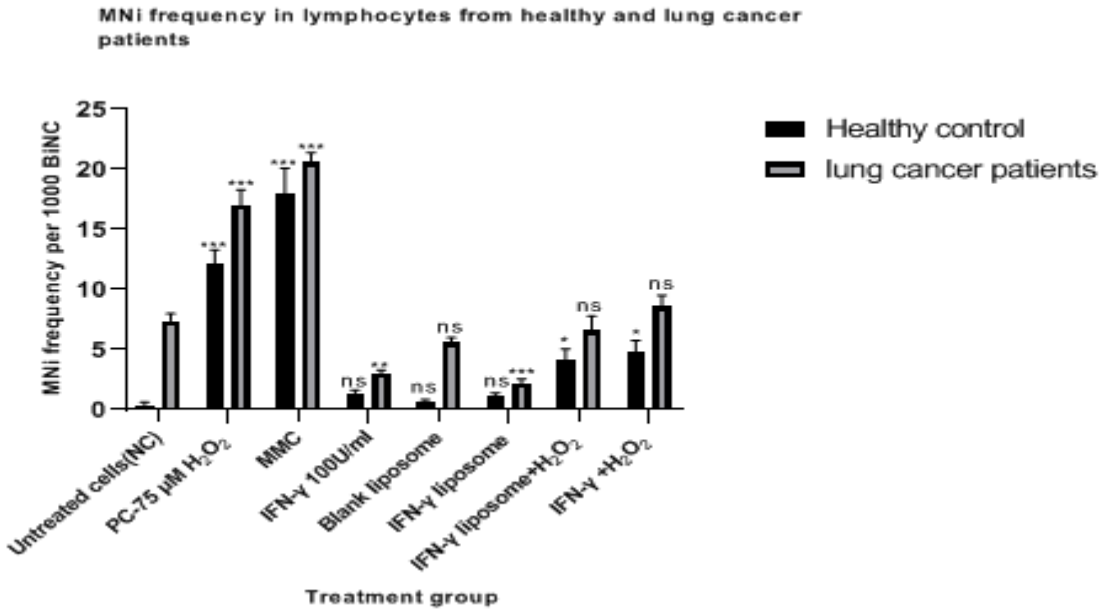

Fig 4: Comparison of MNi frequencies between healthy individuals and lung cancer patients, after treatment with75 μM H_2O_2, 0.4 μM mitomycin c (MMC),100U/ml of IFN-γ B, IFN-γ liposome and 100U/ml of IFN-γ B, IFN-γ liposome in the present of 75 μM H_2O_2. n= 5 (in each group).