VOLUME 03 ISSUE 02 (2024)

AMERICAN JOURNAL OF
LIFE SCIENCE AND INNOVATION (AJLSI)

ISSN: 2833-1397 (ONLINE)

PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

American Journal of Life Science and Innovation (AJLSI)

Volume 4 Issue 1, Year 2024
ISSN: 2833-1397 (Online)
DOI: https://doi.org/10.54536/ajlsi.v4i1.3268
https://journals.e-palli.com/home/index.php/ajlsi

Impact of Bulky & Liposomal Secukinumab on Lymphocytes of Psoriatic Patients, to Determine the Improvement in Bioavailability of Secukinumab Nanolipo

Gazala Layas[1*], Mohammad Isreb[2], Nader Ghaderi[3], Fanila Shahzad[1], Pouria Akhbari[4], Diana Anderson[1], Andrew Wright[3], Mojgan Najafzadeh[1]

Article Information

Received: June 24, 2024

Accepted: July 30, 2024

Published: June 15, 2025

Keywords

Geno Toxicity, Micronucleus Assay, Oxidative Stress, Psoriasis, Secukinumab

ABSTRACT

Psoriasis is an immune-mediated disease affecting the skin and joints. biologic treatments are often used in moderate to severe cases, administered as a monotherapy or in combination with other treatments. Liposomes are versatile and can enhance drug retention, reducing systemic side effects, and are used for therapy and research. This research study aimed to investigate the Geno toxicity of Secukinumab, an ideal biologic treatment for psoriasis (a human IgG1k antibody, anti-IL17A), in bulk and liposome nanoparticles on the lymphocytes of psoriatic patients in comparison with healthy persons. Geno toxicity of Secukinumab in bulk and liposomal form was evaluated and compared by using the Comet and micronucleus assays. From assays, it was demonstrated that Secukinumab in both forms did not exhibit Geno toxicity and reduced DNA damage following the treatment of psoriatic patient lymphocytes and healthy individual's lymphocytes with Secukinumab bulk and liposomes format. Secukinumab used (2.1 and 2.8 μg/mL) with two different concentrations, and effectively decreased DNA damage induced by H_2O_2 in both groups to almost the negative control level. Secukinumab bulk and liposome form markedly reduced the H_2O_2-induced damage and proficiently diminished its adverse effects both in the Comet ($p<0.0001$) and micronucleus as-says ($p<0.01$). Overall, Secukinumab in both forms showed anti-genotoxic and protective effects by expressing its potential to reduce DNA damage produced by oxidative stress and it was observed that it would not induce any further damage in the lymphocytes of healthy individuals and patients.

INTRODUCTION

Psoriasis was initially characterized as a dermatologic disorder primarily affecting epidermal keratinocytes. However, recent studies have shed light on its immune-mediated pathogenesis, making it one of the most prevalent immune-mediated conditions (Kadam *et al.*, 2010). Psoriasis is a chronic autoimmune disease characterized by systemic manifestations and inflammatory changes that predominantly affect the skin and joints.

The disease can significantly compromise the overall quality of life of those affected. Hyper proliferating body patches are primarily caused by autoimmune illness, a disorder marked by an overactive immune system. Psoriasis is a multifaceted disease, and its pathogenesis is not yet fully understood. Despite the availability of several treatment options, psoriasis remains a challenging disease to manage. Therefore, further research is necessary to understand the disease's pathogenesis and develop more effective treatments to improve patients' quality of life (Rendon & Schäkel, 2018). Throughout the world population, up to 2.5% of Psoriasis Vulgaris (chronic skin disorder) prevalence rate was reported (Elder, 2018). Various psoriatic clinical phenotypes are highly regarded as chronic plaque (Psoriasis Vulgaris) with an accountancy of 90% cases (Griffiths & Barker, 2007).

Genetically mutated individuals are believed to have high rate of susceptibility of Psoriasis vulgaris through environmental factors, as a result of which adaptive and innate immune pathways activates inflammatory responses. Lesions are highly visible, directing to a substantial proportion of physical, emotional, social and psychological disability in the patients (Nestle *et al.*, 1994; Chen *et al.*, 2018).

The cause of psoriasis is still unknown. According to a study, erythrocyte-superoxide dismutase activity was lower in psoriasis patients and blood levels of malondialdehyde and nitric oxide end products were greater. The pathogenesis of psoriasis is not fully elucidated; three factors are responsible for the disease pathogenicity including; T-cells, Tumor necrosis factor-alpha (TNFα), and Dendritic cells (Matsuzaki & Umemura, 2018) .

While its major clinical findings are the presence of psoriatic patches on epidermal layer and deep down histologically to the dermis, which revealed that it is not restricted to the epidermal layer. The main member of the IL-17 group, IL-17, interacts with several immune cell types and keratinocytes in the dermal layer of the skin, making psoriasis more pathogenic. There are six components of IL 17 (A-F).

Consequently, the human T cell has number of subsets, and one of these cells is Th17, which produces the cytokine IL-17. The research findings from animal

[1] School of Life Sciences, University of Bradford, Bradford, BD7 1DP, UK
[2] School of Pharmacy, University of Bradford, Richmond Road, Bradford, BD7 1DP, UK
[3] Bradford Teaching Hospitals NHS Foundation Trust, St Luke's Hospital, Little Horton Lane, BD5 0NA, UK
[4] Institute of Biomedical and Clinical Science, College of Medicine and Health, University of Exeter, EX2 5DW, UK
[*] Corresponding author's e-mail: layas698@outlook.com

knockout models proposed that IL-17 and Th17 play a major role to cause the pathogenesis of this disease. By stimulating keratinocyte multiplication in the epidermis, Th17 cytokines IL-17, IL-21, and IL-22, which are released by activated T cells, the adaptive immune system is maintained and psoriatic inflammation is sustained.

IL-17A has been found to be used in the treatment of moderate to severe psoriasis plaque with an improved efficacy. The inhibitory mechanism of IL-17A works swiftly and induces sustained responses with a significant safety assurance profile. Through the clinical severity of the pathological condition, treating parameters of psoriasis are determined, which are based on numerous factors, for instance the dimensions of affected area, the PASI grades, and the Life Quality Index of Dermatology. Mild conditions can be addressed with topical therapies, whereas moderate to severe cases may necessitate phototherapy or systemic therapy, comprising biological or non-biological interventions or a combination of both. Secukinumab, also known as AIN457, is a biological medication that functions as an anti- IL17A monoclonal antibody (mAb). It is employed to treat moderate and severe psoriasis, as well as psoriatic arthritis (Wagener *et al.*, 2013). Chinese Hamster Ovary (CHO) cells were used to manufacture Secukinumab, an IgG1/K-class property (Molden *et al.*, 2021).

Secukinumab has been shown to have a superiority over other biological treatments in terms of achieving severity index PASI 75 (Bagel *et al.*, 2021). Also, it has the ability to sustain its effectiveness over time contrasted to additional biological treatments (Bissonnette *et al.*, 2018). Neutralized and binding aspects of the cytokine interleukin-17A (IL-17A) are responsible to improve the Secukinumab work efficiency for the treatment of psoriasis.

Secukinumab is a fully human mono-clonal antibody that selectively binds and neutralizes the pro-inflammatory cytokine inter-leukin-17A (IL-17A31009130) without producing any effect on IL-17F and Th17 cells (Berg *et al.*, 2021). Additionally, it has no direct impact on the Th1 cascade. Current studies have revealed that increased T-cell abnormalities and oxidative stress (OS) are central parameters that lead to the pathogenesis of psoriasis.

Reactive oxygen species (ROS) suppress regulatory T lymphocytes' (Treg) ability to reduce inflammation while promoting the growth and specialization of Th1/Th17/Th22 cells. Successive secretions of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor (VEGF), interleukin (IL)-17, IL-22, and interferon-gamma (IFN-γ), stimulate keratinocyte number increasing and angiogenesis (Lai *et al.*, 2018; Ciążyńska *et al.*, 2021).

Liposomes are vesicles that contain several thin layered membrane or shells comprised of a phospholipid bilayer covering an insignificant amount of aqueous liquid, used for biotechnological and biomedical roles. On the basis of preparation and its use, they are different in size ranging from several specific nanometers to hundred micrometers (Lombardo & Kiselev, 2022). Bio-logically active compounds of liposomes, such as genetic materials, chelating agents, proteins, enzymes, peptides, vaccines, hormones and antimicrobial agents have been evaluated for patient use.

Because of its superior mechanical, optical, and electrical capabilities, quantum mechanics, and nanoscale volume-surface area ratio, liposomes are becoming more and more common in sophisticated technology (Sercombe *et al.*, 2015; Nsairat *et al.*, 2022). Because they increase the flow of drugs, they also aid in reducing treatment side effects.

This study aimed to determine that how liposomes and Secukinumab bulk form affected DNA damage in peripheral blood cells from both healthy people and psoriasis sufferers by using the Comet assay and Micronucleus (CBMN) assay. Additionally, the study aimed to determine the best concentration of

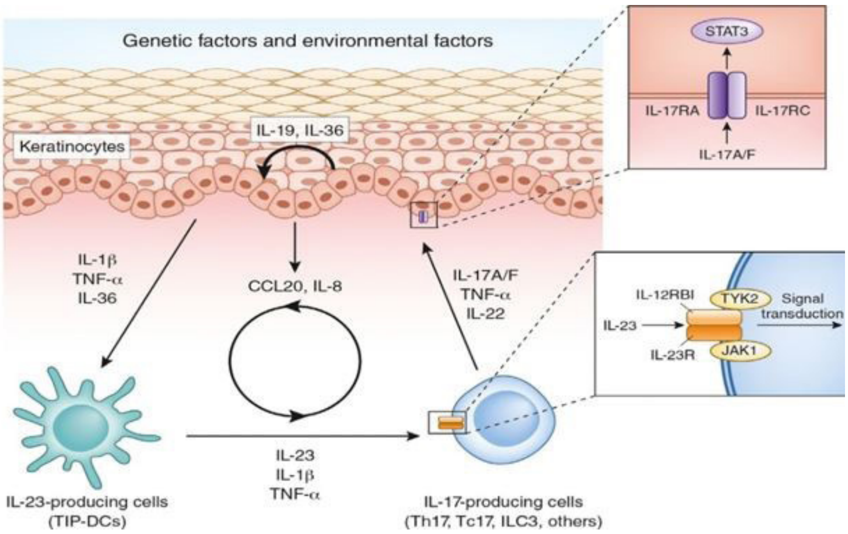

Figure 1: Pathogenesis of psoriasis, the cytokines were activated in psoriasis and their further cascades in the skin. Interleukin (IL)-23 produced by various IL-23- producing cells induces IL-17 production from various IL-17-producing cells such as T-helper (Th)17, IL-17 producing CD8+ T cells (Tc17), and innate lymphoid cells type 3 (ILC3). IL-17 stimulates keratinocytes to produce inflammatory cytokines/chemokines, which further activate IL-23-producing cells and recruit IL-17-producing cells and neutrophils

Secukinumab liposome to prepare topically for treating psoriasis in the future.

The cellular response to the bulk and liposome forms was investigated and the optimal dose for bulk and liposome forms was obtained. In the presented study, the Geno toxicity assays with different end-points were used to assess the impact of Secukinumab bulk and liposome forms on lymphocytes from psoriasis patients contrasted to healthy controls (Yamanaka, 2021).

MATERIALS AND METHODS

Materials

Secukinumab was obtained from Novartis Pharmaceuticals (Cosentyx). 1, 2-Dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DPPG), 1,2-dioleoyl-sn-glycero-3-phsphoethanolamine (DOPE), purity >99%, Cholesterol (Chol), Methanol, Dichloromethane (DCM) were obtained from Sigma-Aldrich Company Ltd. (UK). All other reagents used were derived from Sigma Aldrich U.K. unless otherwise stated. For the preparation of Secukinumab solutions, drugs were in distilled water (150mg/ml). A 75µg/ml stock solution was prepared. The prefilled Secukinumab syringes (150 mg) were used in this study to produce the liposome format of this biological agent and investigate its comparison with bulk form. The compound was diluted to a suitable concentration for treating the cells in vitro.

Characterization and Production of Peptide-Loaded Liposome

Liposomes were prepared by using the Bangham process, which involved dissolving methanol and Dichloro-methane (DCM) and methanol (3:1 v/v) with1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt DPPG, and (2 mg), 1,2-dioleoyl-sn-glycero-3-phsphoethanolamine DOPE, and cholesterol (2:2:1 molar ratio). The solution was transmitted to a rotary evaporator flask and at 40 °C the respective organic solvent mixture and after six hours of evaporation, the mixture was rehydrated with 10ml distilled water.

After that, the films were placed within 1ml of Secukinumab for 1 h at 60 °C by employing a bath Sonicator (150 W), then the sample exposed through freeze-thaw cycles at -20°C and thawing at 60°C, and then diluted with 75 µg/ml of distilled water. Centrifuging the finished mixture at 22,000 x g for 30 minutes at 4 °C, helped to guarantee purification and eliminate any remaining non-encapsulated medicines.

The modest size and Poly Disparity index of the liposome formulations were evaluated by means of dynamic light scattering (DLS) with Zetasizer ZS-90 and Model ZEN 3600. In triplicate form, all measurements were performed. Measures were found to be < 150 nanometers.

Ethical Approval

The study on the genetic and environmental effects on lymphocytes from different illness situations, such as inflammatory, malignant, and precancerous ones, employing different genetic endpoints, has been authorized by the Leeds East Research Ethics Committee (rec) (rec reference number: 12/YH/0464). Ethical approval has been given by the subcommittee for Ethics in Research with Human Subjects at the University of Bradford (Reference no.: 0405/8). The Research Support and Governance Office Bradford Teaching Hospital NHS Foundation granted the Re DA number: 1202.

Blood Sample Collection

Blood samples were collected from 40 individuals (20 healthy and 20 patients) using the vein-puncture technique, after obtaining consent and following safety measures. Basic information was gathered from individuals about health and lifestyle factors using a questionnaire, as shown in Table. 1, while healthy individual's data was presented in Table. 2. Blood was collected from a patient with moderate Psoriasis under systemic treatment; non were taking any biological treatment.

Table 1: List of patients' confounding factors

Confounding Factors	Categories	Frequencies (n=20)	Percentage (n=20)
Age	21-40	6	30
	41-60	7	35
	60-80	7	35
Gender	Male	9	45
	Female	11	55
Ethnicity	Asian	3	15
	Caucasian	17	85
Smoking history	Ex-Smoker	5	25
	Non-Smoker	15	75

Table 2: List of healthy controls confounding factors

Confounding Factors	Categories	Frequencies (n=20)	Percentage (n=20
Age	20-35	14	70
	36-50	6	30
Gender	Male	10	50
	Female	10	50
Ethnicity	Asian	10	50
	Caucasian	7	35
	African	1	5
	Arab	2	10
Smoking history	Non Smoker	19	95
	Ex-Smoker	1	5

Cell Viability Determination

Cell viability was determined using the cell counting kit 8 (CCK8) from VWR, UK. Blood samples were added with the chemicals in Eppendorf tubes as described earlier for the Comet assay treatment step and RPMI-1640 medium for the preparation of cell culture. After that tubes were subjected to centrifugation at 3000 rpm (705 g) and the remained precipitates were discarded, and the mass of cells were suspended. Following the manufacturer's procedure, the experimental strategy was carried out and the kit's contents were applied to the cell suspension. Cell viability of ≥80% was considered for use in all experiments.

Cell Treatment and the Comet Assay

Complete blood samples from 20 psoriasis patients and 20 healthy persons were collected and supplemented with 10% DMSO and RPMI medium and quickly stored at -80 °C. Five groups were selected for treatment, in order to conduct the experiment, including an un-treated group (NC) containing the blood sample and RPMI 1640 medium. A positive control containing media, blood, and a total of 10µl, 75µM/ml Hydrogen Peroxide (H_2O_2), and two treatment groups with different doses of "Secukinumab (2.8µg /ml, 2.1µg/ml) including or excluding H_2O_2", and 1000µl of treatment volume were used in the experiment. It was then "centrifuged at 3000 rpm" and incubated at 37°C (Tice *et al.*, 2000; Najafzadeh & Anderson, 2016).

Using a computer terminal Komet 6 software and Kinetic Imaging (Andor Technology Ltd, Belfast) and fluorescence microscope (20X magnification) equipped with CCD camera, 100 cells from each treatment group were fixed using Comet assay parameters such as Olive tail moment and percentage tail DNA.

Cytokinesis Block Micronucleus Assay (CBMN)

Fresh blood sample (350 µl) and 130µl of phytohaemagglutinin (PHA) were added to 25cm^3 vented cap corning flasks containing "4.5 ml RPMI-1640 medium augmented with 1% of Penicillin-Streptomycin (Aminoglycoside antibiotic) and 15% Fetal Bovine Serum (FBS)". Followed by incubation for 24h at room temperature i.e., 37^{0}C in the existence of 5% CO_2 and then the test chemicals Secukinumab bulk and liposome, H_2O_2 were added. For the untreated negative control plain basic medium was used, and as positive controls Mitomycin C (MMC) (0.4µM) and H_2O_2, (75µM) were used. In each flask 30µl of Cytochalasin B (cyt B) (1mg/ml) was added at 44h and cultured under the same conditions for another 20h (Fenech *et al.*, 2016).

Using a 40X microscope to score 1000 cells per treatment group, including the Nuclear Division Index and the percentage of binucleated cells, the frequency of Micro Nuclei (MNi) was ascertained (Fenech, 2007). The NDI was calculated using the Equation

Eq. A.1: NDI = M1 + 2(M2) + 3(M3) / N

Where, M1 = mononucleated cells, M2= binucleated cells, M3 = multinucleated cells, N = total number of viable cells scored (Fenech *et al.*, 2016).

Statistical Analysis

For the determination of significant values, data was analyzed through one-way ANOVA (tofind the difference between results obtained from research) and t-tests to determine the significant values. A value P= <0.05 was considered statistically significant. Graph Pad Prism 8 was used for statistical data analysis.

RESULTS AND DISCUSSION

Results

When Secukinumab liposomes were TEM-analyzed, homogeneous, spherical bio particles with sizes between 100 and 200 nm were discovered.

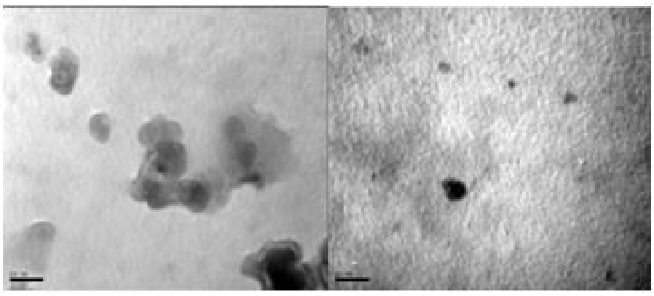

Figure 2: (a) TEM analysis of the aqueous dispersion of Secukinumab, (b) TEM imaging the Liposomal form of Secukinumab

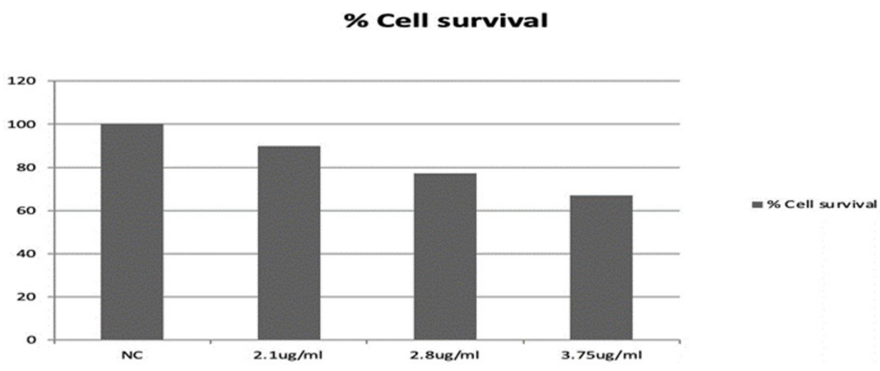

Figure 3: Cytotoxicity of Secukinumab on human peripheral lymphocytes after 24 hrs exposure to different concentrations (2.1, 2.8, and 3.751 µg/mL) by using CCK8

The proliferation rate was computed by dividing the total number of counted cells by the number of living or viable cells. A cell counting kit was used to check the integrity and viability of the lymphocytes, as shown in Figure 3.

The Effects of Secukinumab and H_2O_2 on the Lymphocytes from Healthy

The responses of bulk and liposome forms of Secukinumab on lymphocytes of DNA from healthy and psoriatic patients treated with H_2O_2 using "Olive Tail Moment" (OTM), standard errors and significance were displayed in the Figure 4. Using one-way ANOVA, the study showed that H_2O_2, treatments significantly increased DNA damage in cells by increasing OTM **$p \leq 0.01$in psoriatic and ***$p \leq 0.001$ in healthy individuals in comparison to untreated cells. However, Secukinumab treatments in the two different forms with or without H_2O_2 were not significant (ns).

In comparison with the positive control treatments, a remarkable reduction in the DNA damage was observed after the addition of Secukinumab ***$p \leq 0.001$ for the bulk in patients without the H_2O_2, and **$p \leq 0.01$ for all the other treatments, as shown in Figure 4.

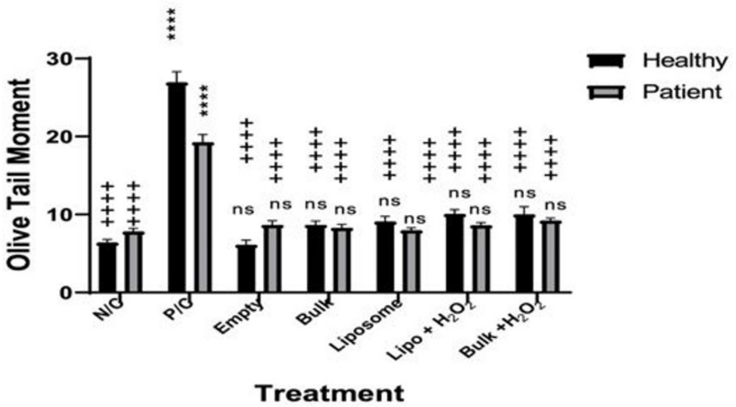

Figure 4: The effects of Secukinumab and H_2O_2, on the lymphocytes from healthy and patient groups measuring OTM (* com-paring the treatments to the without treatments or negative control or NC, ns (non-significant) comparing the groups with different treatments to the NC group, + comparing the groups with different treatments to the positive control or PC) (N=20 in the healthy control group and 20 in the patient group),), N/C stands for the negative control, P/C; positive control, Bulk; Secukinumab bulk, liposome (liposome form of Secukinumab), empty; liposomes with no drug, hydrogen peroxide (H_2O_2)

Results presented in Figure 4 illustrate the responses of bulk and liposome forms of Secukinumab on lymphocyte DNA from healthy and psoriatic individuals treated with H_2O_2 using Tail DNA %, standard errors SE and significance. DNA damage observed in the patient group was higher compared to the healthy as predicted. The PC revealed a significant rise in the Tail DNA control cells, ****$p \leq 0.0001$. In comparison to the NC, Secukinumab treatments—liposomal and bulk, with or without H_2O_2, were not very important. When compared to PC therapies, the inclusion of Secukinumab ++++$p \leq 0.000$, considerably reduced the damage, as shown in Figure 5.

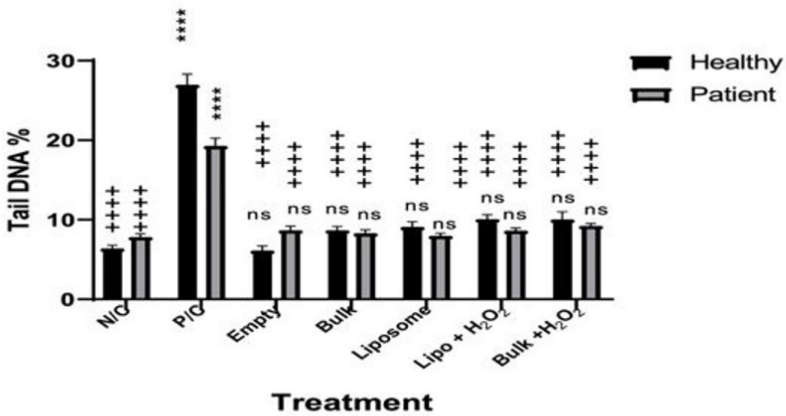

Figure 5: The effects of Secukinumab and H_2O_2 on the lymphocytes from 20 healthy and 20 patient groups measuring Tail DNA% (* comparing the treatments to the NC, ns (non-significant) comparing the treatments to the NC, + comparing the treat-ments to the PC) (N=20 in the healthy control group and 20 in the patient group),), N/C stands for the negative control, P/C; positive control, Bulk; secukinumab bulk, liposome (liposome form of secukinumab), empty; liposomes with no drug, hydrogen peroxide (H_2O_2).

Cytokinesis Block Micronucleus Assay (CBMN)
The micronucleus assay reveals higher levels of MNis in patients who are not receiving therapy, making it a dependable and appropriate approach for examining DNA damage at chromosomal levels. Secukinumab 2.1µg/ml in bulk and liposome forms has shown some attenuation of the consequences caused by H_2O_2, as illustrated in Figure 6.

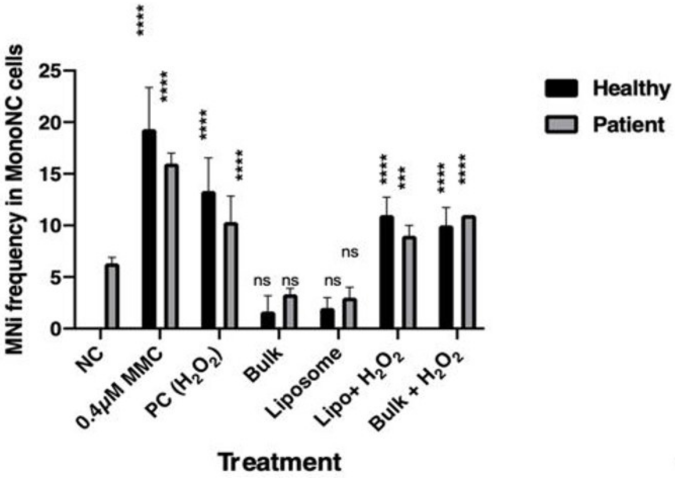

Figure 6: The average of MNi in BiNC scored per 1000 cells from 5 healthy individuals and 5 psoriasis patients, n=1000. Data are expressed as means ± standard error (SE).Seven treatment groups included the negative control, two positive control groups (0.4µM MMC) (75µM of H_2O_2), 2.1µg/ml of Secukinumab, 2.1µg/ml of Secukinumab with H_2O_2 and the liposome form of both (*** represents P< 0.0001, **p<0.001, *p<0.02, *p<0.018, ns=not significant). (N=5 in the healthy control group and 5in the patient group),), N/C stands for the negative control, P/C; positive control, Bulk; Secukinumab bulk, liposome (liposome form of Secukinumab), empty; liposomes with no drug, hydrogen peroxide (H_2O_2)

Discussion
Secukinumab currently is one of the best choices with proper efficacy on psoriasis conditions, even on the increased level of IL-23 and JAK inhibitors. However, in the case of Secukinumab failure, combined therapy would be ideal (Damiani *et al.*, 2022). Also, Secukinumab is a well-known biological therapy considered the most cost-effective bio-logical agent (Schweikert *et al.*, 2020) .
Moreover, a range of side effects linked with these biological compounds, such as paradoxical reactions (PRs), which are the result of worsening of immune-mediated inflammatory disease (Miyagawa, 2022) and increased eosinophilic disorders (Bridgewood *et al.*,, 2022). It is therefore desirable to reduce the con-centration of the antibody in the blood and increase its residency in the psoriatic dermal layer by encapsulation in liposomes. Using the Comet and CBMN assays, the study examined the effects of "Secukinumab bulk and liposome forms" on human peripheral lymphocytes from 20 psoriasis patients and 20 healthy persons.
The research examined Secukinumab's possible protective effects on H_2O_2-induced DNA damage in lymphocytes, demonstrating the anti-oxidant properties of both forms and damage that was similar to that of patients who were not treated. Our results showed that Secukinumab significantly de-creased the percentage of DNA damage in lymphocytes in the Comet assay (+++ p < 0.001).
Both groups had considerable levels of DNA damage, according to the Comet assay, but Secukinumab dramatically reduced the effects of H_2O_2 in both liposomal and bulk forms. The investigation, which used both versions of Secukinumab, supplemented with or without H_2O_2, to treat patient groups and healthy individuals, did not find any statistically significant differences in DNA damage.
Empty liposomes had no significant damage on the cell, which shows that it in itself is safe on cells. According to the study, Secukinumab may be able to both protect and lessen the damage that H_2O_2 causes to DNA in both healthy people and psoriasis patients.
Oxidative stress-induced synthesis and amplification of pro-inflammatory cytokines can be inhibited by the anti-IL-17 drug Secukinumab (Zou & Meng, 2021). Increased reactive oxygen species (ROS) damage DNA, lipids, and proteins, as well as signaling molecules and other physiological activities, leading to diseases through oxidative stress.
When compared to the negative control, the oxidative stress therapy Secukinumab did not significantly damage DNA in lymphocytes from either patient group or from healthy individuals. The outcomes were very similar to those of the negative control. This suggests that the concentration of 2.1µg/ml of Secukinumab is non-genotoxic to the cells in both groups.
Hydrogen peroxide is an oxidative stress inducer compound which causes significant amounts of oxidative stress-related DNA damage in peripheral lymphocyte cells (Najafzadeh *et al.*, 2009; Stanić *et al.*, 2016). Our results are in accordance with these studies as H_2O_2 significantly induced DNA damage in both subjective groups; healthy individuals and psoriasis patients. When compared to the positive control alone, the administration of various

forms of Secukinumab, such as liposome and bulk form, dramatically decreased DNA damage caused by H_2O_2. Secukinumab, with antioxidant defense playing a part, reduced damage to a level comparable to the untreated control group. The Psoriasis Area and Severity Index and oxidative stress indicators were found to be positively correlated.

Using CCK8, the study examined the cytotoxic effects of Secukinumab on healthy peripheral lymphocytes, and the results showed that these cells were cytotoxic in a concentration-dependent manner (Lin & Huang, 2016). It was observed that, increased concentration of Secukinumab (3.75µg/ml) was found to be cytotoxic, while the two lower concentrations of the drugs were tested 2.1µg/ml and 2.8µg/ml, had increased cell survival rates of over 75%.

The CBMN assay is an effective test to study the capability of genotoxic agents to cause various clastogenic (chromosome breakage) and an eugenic effects (causing daughter cells to have an abnormal number of chromosomes during cell division) (Fenech, 2009). It was utilized in the current study to describe the effects of Secukinumab on healthy individuals versus psoriasis patients. Also, to determine the protective potential of the drug against H_2O_2-induced Geno toxicity. This assay assesses several parameters, including micronuclei (MNi), which form during anaphase and indicate chromosomal remains or lost chromosomes generated during nuclear division.

To enhance the sensitivity and reliability of the assay, cytokinesis is blocked using cytochalasin B, which facilitates the accumulation of BiNC. The presence of MNi in BiNC only reflects damage induced after treatment, reducing the possibility of scoring pre-existing damage. This approach effectively determines the effects of the test chemicals. By implementing this methodology, the sensitivity and reliability of the assay are increased, and the effects of the test chemicals are accurately determined (Magdolenova *et al.*, 2012; Co-operation OfE, 2010).

In comparison with the untreated cells, no effects on MNi frequency were reported when the lymphocytes of healthy patients receiving both kinds of Secukinumab treatment.

"The MNi frequency in lymphocytes(***$p<0.001$) was considerably elevated by the MMC and 75 µM H_2O_2, yet Secukinumab (bulk or liposome co-supplemented with H_2O_2) exhibited significant reduction in the number of MNi in lymphocytes (*$p<0.05$)".

"It was manifested that liposomal Secukinumab caused a significant reduction in the number of MNis in patients (***$p<0.001$ as compared to un-treated cells). Moreover, the inclusion of MMC and 75 µM H_2O_2 revealed a significant increase (***$p<0.001$) in the MNi number in lymphocyte count in comparison to untreated cells". This all confirms the safety of Secukinumab in both forms, bulk and liposome, on lymphocytes. This could be the first step in using the liposome form as a topical treatment.

Therefore, the results demonstrate that all the treatment sets from both investigative groups have shown the BiNC % and the NDI within the normal ranges: a typical NDI value represents a successful division. Evaluation of the assays shows that treatment of healthy and patient groups with Secukinumab in both forms did not induce a significant number of MNi in BiNC at basal levels. However, when 2.1µg/ml of the drug was co-treated with H_2O_2, Secukinumab provided substantial protection against H_2O_2-induced damage and reduced the frequency of MNi in healthy BiNC.

A significantly raised number of MNi were observed in Mono NC of the patient group compared to healthy individuals, which indicates pre-existence of DNA and chromosomal damage due to the disease state, medications or other confounding factors. Secukinumab 2.1µg/ml re-duced the frequency of MNi in MonoNC of the patient group at a basal level. However, the results were not statistically significant. The consistency of the data suggests that con-founding factors were not an issue.

Limitations

- ➢ Due to differing degrees of safety and efficacy, standardizing liposomal and bulk Secukinumab formulations and dosages is essential to maintaining similar results throughout investigations.
- ➢ The liposomal and bulk medication Secukinumab may protect against oxidative stress, but more research is necessary to assess any possible off-target effects and determine the treatment's overall risk-benefit profile.

CONCLUSION

The aim of this study was to investigate the effect of Secukinumab, in both liposomal and non-liposomal forms, on DNA damage in healthy individuals and psoriasis patients. Despite higher levels of damage in the sick group compared to the healthy group, Secukinumab significantly reduced H_2O_2-induced DNA damage in the presence of H_2O_2, attenuating its harmful effects.

The results were consistent across the Comet and micronucleus assays, with Secukinumab 2.1µg/ml demonstrating enhanced efficacy against H_2O_2-mediated DNA damage. According to the study, both types of Secukinumab have the ability to protect against oxidative stress caused by H_2O_2, which may help with repair without having a major negative impact on Geno toxicity. Furthermore, the liposomal form of the drug was found to be safe for localized treatment via subcutaneous injection or transdermal drug delivery preparation. However, additional investigations are required to elucidate other possible molecular mechanisms involved.

In conclusion, this study demonstrated the ability to effectively deliver Secukinumab via liposomes, which may have clinical applications. These results highlight the potential of Secukinumab to serve as a protective agent against oxidative stress-induced DNA dam-age. Overall, this study provides valuable insight into the effects of Secukinumab on DNA damage and its potential clinical applications.

Recommendations

➢ Extensive research ought to appraise Secukinumab's enduring impacts on oxidative stress and Geno toxicity in order to appraise its resilience and possible unfavorable consequences in the long run.

➢ Large-scale clinical trials involving human participants are required for further research studies to evaluate Secukinumab's efficacy, safety, and tolerability in a range of patient populations.

Acknowledgement

Authors are thankful to University of Bradford, for their continued support in this research work.

Authors Contribution

All authors contributed to the study conception and design. G. Layas & M. Isreb N. Ghaderi & Fanila: Conceptualization, Methodology including ethica approval, samples collections and statistical analysis; P. Akhbari, D. Andreson: Analytical interpretation; A. Wright & M. Najafzadeh: Research discussion and review.

Abbreviations

CHO Chinese Hamster Ovary cells
Chol Cholesterol
CBMN cytokinesis-block micronucleus
DCM methanol dichloromethane
DOPE Dioleoyl phsphoethanolamine
DPPG 1,2-Dipalmitoyl-sn-glycerol sodium salt
IL-17 Interleukin 17
mAb Monoclonal antibody
PSAI Psoriasis Area and Severity Index
Th T-Helper cells
TNFa Tumor necrosis factor-alpha
RPMI Mammalian cell culture media/ Roswell Park Memorial Institute

REFERENCES

Bagel, J., Blauvelt, A., Nia, J., Hashim, P., Patekar, M., De Vera, A. (2021). Secukinumab maintains superiority over ustekinumab in clearing skin and improving quality of life in patients with moderate to severe plaque psoriasis: 52-week results from a double-blind phase 3b trial (CLARITY). *Journal of the European Academy of Dermatology and Venereology, 35*(1), 135–142.

Berg, S. H., Balogh, E. A., Ghamrawi, R. I., & Feldman, S. R. (2021). A review of secukinumab in psoriasis treatment. *Immunotherapy, 13*(3), 201–216.

Bissonnette, R., Luger, T., Thaçi, D., Toth, D., Lacombe, A., Xia, S. (2018). Secukinumab demonstrates high sustained efficacy and a favourable safety profile in patients with moderate‐to‐severe psoriasis through 5 years of treatment (SCULPTURE Extension Study). *Journal of the European Academy of Dermatology and Venereology, 32*(9), 1507–1514.

Bridgewood, C., Wittmann, M., Macleod, T., Watad, A., Newton, D., Bhan, K. (2022). T helper 2 IL-4/ IL-13 dual blockade with dupilumab is linked to some emergent T helper 17–type diseases, including seronegative arthritis and enthesitis/enthesopathy, but not to humoral autoimmune diseases. *Journal of Investigative Dermatology, 142*(10), 2660–2667.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget, 9*(6), 7204–7218.

Ciążyńska, M., Olejniczak-Staruch, I., Sobolewska-Sztychny, D., Narbutt, J., Skibińska, M., & Lesiak, A. (2021). Ultraviolet radiation and chronic inflammation—Molecules and mechanisms involved in skin carcinogenesis: A narrative review. *Life, 11*(4), 326.

Damiani, G., Odorici, G., Pacifico, A., Morrone, A., Conic, R. R., Davidson, T., *et al.* (2022). Secukinumab loss of efficacy is perfectly counteracted by the introduction of combination therapy (rescue therapy): Data from a multicenter real-life study in a cohort of Italian psoriatic patients that avoided secukinumab switching. *Pharmaceuticals, 15*(1), 95

Elder, J. T. (Ed.). (2018). Expanded genome-wide association study meta-analysis of psoriasis expands the catalog of common psoriasis-associated variants. *Journal of Investigative Dermatology Symposium Proceedings.* Elsevier.

Fenech, M., Knasmueller, S., Bolognesi, C., Bonassi, S., Holland, N., Migliore, L., *et al.* (2016). Molecular mechanisms by which in vivo exposure to exogenous chemical genotoxic agents can lead to micronucleus formation in lymphocytes in vivo and ex vivo in humans. *Mutation Research/Reviews in Mutation Research, 770,* 12–25.

Fenech, M. (2007). Cytokinesis-block micronucleus cytome assay. *Nature Protocols, 2*(5), 1084–1104.

Fenech, M. (2009). A lifetime passion for micronucleus cytome assays—reflections from Down Under. *Mutation Research/Reviews in Mutation Research, 681*(2-3), 111–117.

Griffiths, C. E., & Barker, J. N. (2007). Pathogenesis and clinical features of psoriasis. *The Lancet, 370*(9583), 263–271.

Kadam, D. P., Suryakar, A. N., Ankush, R. D., Kadam, C. Y., & Deshpande, K. H. (2010). Role of oxidative stress in various stages of psoriasis. *Indian Journal of Clinical Biochemistry, 25,* 388–392.

Lai, R., Xian, D., Xiong, X., Yang, L., Song, J., & Zhong, J. (2018). Proanthocyanidins: Novel treatment for psoriasis that reduces oxidative stress and modulates Th17 and Treg cells. *Redox Report, 23*(1), 130–135.

Lin, X., & Huang, T. (2016). Oxidative stress in psoriasis and potential therapeutic use of antioxidants. *Free Radical Research, 50*(6), 585–595.

Lombardo, D., & Kiselev, M. A. (2022). Methods of liposomes preparation: Formation and control factors of versatile nanocarriers for biomedical and nanomedicine application. *Pharmaceutics, 14*(3), 543.

Matsuzaki, G., & Umemura, M. (2018). Interleukin-17 family cytokines in protective immunity against infections: Role of hematopoietic cell-derived and non-hematopoietic cell-derived interleukin-17s. *Microbiology and Immunology, 62*(1), 1–13.

Magdolenova, Z., Lorenzo, Y., Collins, A., & Dusinska, M. (2012). Can standard genotoxicity tests be applied to nanoparticles? *Journal of Toxicology and Environmental Health, Part A, 75*(13-15), 800–806.

Miyagawa, F. (2022). Pathogenesis of paradoxical reactions associated with targeted biologic agents for inflammatory skin diseases. *Biomedicines, 10*(7), 1485.

Molden, R., Hu, M., Yen, E. S., Saggese, D., Reilly, J., Mattila, J. (Eds.). (2021). Host cell protein profiling of commercial therapeutic protein drugs as a benchmark for monoclonal antibody-based therapeutic protein development. *mAbs, 13*(1), 1901684.

Najafzadeh, M., & Anderson, D. (2016). The use of isolated peripheral lymphocytes and human whole blood in the comet assay. *Comet Assay in Toxicology,* 177–191.

Najafzadeh, M., Gueidan, C., Badali, H., Van Den Ende, A. G., Xi, L., & De Hoog, G. (2009). Genetic diversity and species delimitation in the opportunistic genus Fonsecaea. *Medical Mycology, 47*(1), 17–25.

Nestle, F., Turka, L., & Nickoloff, B. (1994). Characterization of dermal dendritic cells in psoriasis: Autostimulation of T lymphocytes and induction of Th1 type cytokines. *The Journal of Clinical Investigation, 94*(1), 202–209.

Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A., & Alshaer, W. (2022). Liposomes: Structure, composition, types, and clinical applications. *Heliyon, 8*(2), e08829.

Organisation for Economic Co-operation and Development (OECD). (2010). *Test No. 487: In vitro mammalian cell micronucleus test.* OECD Publishing.

Rendon, A., & Schäkel, K. (2019). Psoriasis pathogenesis and treatment. *International Journal of Molecular Sciences, 20*(6), 1475.

Sercombe, L., Veerati, T., Moheimani, F., Wu, S. Y., Sood, A. K., & Hua, S. (2015). Advances and challenges of liposome assisted drug delivery. *Frontiers in Pharmacology, 6,* 286.

Schweikert, B., Malmberg, C., Åkerborg, Ö., Kumar, G., Nott, D., Kiri, S. (2020). Cost-effectiveness analysis of sequential biologic therapy with ixekizumab versus secukinumab in the treatment of active psoriatic arthritis with concomitant moderate-to-severe psoriasis in the UK. *PharmacoEconomics - Open, 4*(4), 635–648.

Stanić, D., Plećaš-Solarović, B., Petrović, J., Bogavac-Stanojević, N., Sopić, M., Kotur-Stevuljević, J., *et al.* (2016). Hydrogen peroxide-induced oxidative damage in peripheral blood lymphocytes from rats chronically treated with corticosterone: The protective effect of oxytocin treatment. *Chemico-Biological Interactions, 256,* 134–141.

Tice, R. R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., ... & Sasaki, Y. F. (2000). Single cell gel/comet assay: Guidelines for in vitro and in vivo genetic toxicology testing. *Environmental and Molecular Mutagenesis, 35*(3), 206–221.

Wagener, F. A., Carels, C. E., & Lundvig, D. M. (2013). Targeting the redox balance in inflammatory skin conditions. *International Journal of Molecular Sciences, 14*(5), 9126–9167.

Yamanaka, K. (2021). Skin disease and comorbidities. *MDPI, 11*(8), 5754.

Zou, Y., & Meng, Z. (2021). Literature overview of the IL-17 inhibition from psoriasis to COVID-19. *Journal of Inflammation Research, 14,* 5611–5621.