Anti-cancer effect of a novel formulation from CBSC-derived exosomes And Retinoic acid in the treatment of Melanoma

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Background

Malignant Melanoma is a serious form of skin cancer that begins in cells known as melanocytes. It is the 5th most common cancer in the UK, accounting for 4% of all new cancer cases (2016-2018). An estimated 60-70% of Malignant Melanoma are caused by ultraviolet (UV) radiations exposure. Previous research shows that the UV rays that damage skin can also alter a P53 gene that suppresses tumours, raising the risk of sun-damaged skin cells developed into skin cancer. The treatment of malignant melanoma is a challenge for clinicians because of its aggressive behaviour and metastatic status. In the last 20 years, Retinoid therapy have produced remarkable results in the treatment of malignant melanoma. The retinoid therapy mainly focus on the inhibition of carcinogenesis by apoptosis. Recently, extracellular vesicles, especially exosomes, have been highlighted for their therapeutic benefits in numerous chronic diseases. Exosomes display multifunctional properties, including inhibition of cancer cell proliferation and initiation of apoptosis. This invitro study investigated the combined effect of Retinoic acid and CBSC-derived exosomes as anti-cancer therapy.

Objective

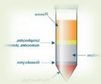
The aim of the study is to investigate Retinoic acid (RA) and Umbilical cord derived stem cells, as a potential novel treatment for melanoma. The objective is to investigate the effects of RA and exosomes on the lymphocytes of healthy individuals and melanoma patients' lymphocytes and to the detect anti-cancer activity of RA and exosomes on CHL-1 melanoma cell line either alone or in combination of both as compared to untreated cells using the Comet assay and CCK8 assay.

Methodology

Patients and Study Design

A) Isolation Of Lymphocytes From Healthy Individuals

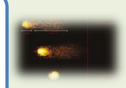
B) Lymphocytes From Melanoma Patients



Cell Culture Of CHL-1 Cell Lines

Cytotoxicity

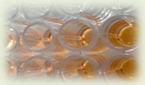
CCK8 On Lymphocytes From Healthy And On Melanoma Cell Line With Different Concentration Of Retinoic acid For 24h And 48h



The Comet Assay Was Performed To Detect The Level Of DNA Damage (OTM And % TDNA) With Best Doses Of CBSC-Exosome After Performing CCK8 After 48h

Cytotoxicity

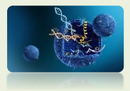
CCK8 On Lymphocytes From Healthy And On Melanoma Cell Line With Different Concentration Of CBSC-Exosome For 3h,12 24h And 48h



The Comet Assay Was Performed To Detect The Level Of DNA Damage (OTM And % TDNA) With Best Doses Of Retinoic acid After Performing CCK8 After 48h

CBSC-Exosome Preparation

2mg Of CBSC-Exosome In 5ml Of Bps

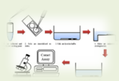


The Comet Assay Was Performed To Detect The Level Of DNA Damage (OTM And % TDNA) CHL-1 Cell Line With The Retinoic acid

N=3

The Comet Assay Was Performed To Detect The Level Of DNA Damage (OTM And % TDNA) CHL-1 Cell Line With The CBSC-Exosome

N=3



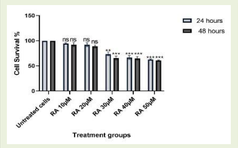
Results

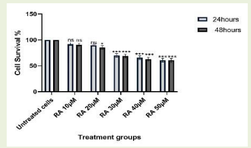
- The Hydrogen peroxide and ultraviolet A+B light (315-350nm) were used to cause oxidative stress. A concentration of RA 10µMand 20µMl were used to treat the lymphocytes in the comet assays.
- The lymphocytes from melanoma patients showed increased DNA damage as compared to healthy individuals (*p<0.05). There was no damage observed in the healthy lymphocytes, but it produced significant (***p<0.001) reduction in the DNA damage of melanoma lymphocytes in the comet assay.
- Moreover, the RA 10µM significantly decreased the oxidative stress caused by hydrogen peroxide and UVA+B rays. Hence, RA is effective in both groups using the Comet assay.
- The Comet assay results depicted significant DNA damage in a melanoma cell line (CHL-1) treated with Retinoic acid and exosomes, both the cytotoxicity of CHL-1 treated with Retinoic acid and exosomes exhibited a significant time-dependent decrease in cell survival.
- The combination treatment of both RA and exosomes was also tested in CHL-1 melanoma cell line. The results showed that DNA damage was elevated in CHL-1 cells when treated with the combination treatment (10µM + 120µM) as compared to both treatment alone

Statistical Analysis

To analyse the Comet assay and CCK8 assay results as Mean±SEM, one-way ANOVA and Dunnett's post hoc test was used and all histograms which includes error bars were statistically significant at level (*p<0.05, **p<0.01 and ***p<0.001).

A)





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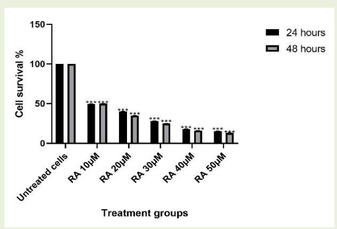
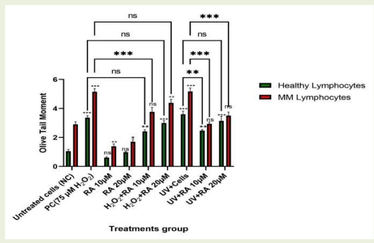


Figure 1 Retinoic Acid caused a significant time and dose dependent decrease in the cell survival rate of CHL-1 Cells and lymphocytes got from melanoma patient compared to healthy lymphocytes. A) Lymphocytes from malignant melanoma patients (LMM) and lymphocytes from healthy individuals were incubated separately with different concentration of 10, 20, 30, 40 and 50µM of RA for 24 and 48 hrs. B) CHL-1 Melanoma cells were incubated for 24 and 48 hrs with the same concentrations of RA (n=3). Cell proliferation analysis was performed by cck8 assay. Error bars represent SEM. *P <0.05, ***P <0.001, **P<0.01. Data was compared with the untreated cells and analysed by one way ANOVA.

A)



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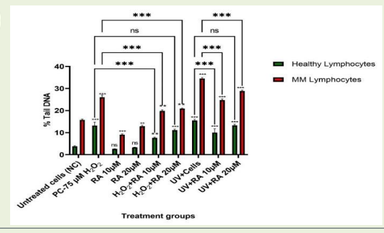
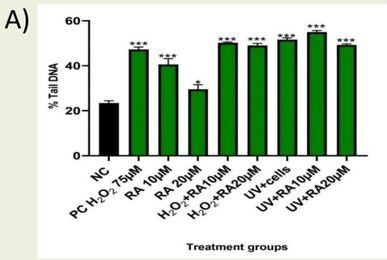


Figure 2 Both A and B compares the concentration-response of 10µM and 20µM RA with 75µM hydrogen peroxide on lymphocytes from 20 healthy and 20 melanoma patients using %Tail DNA and olive tail moment. The graph explained that lymphocytes from melanoma patients showed more DNA damage than healthy lymphocytes. It also explained that in treated lymphocytes from healthy blood with RA 10µM and 30µM with 75µM H202, the DNA damage in the lymphocytes was reduced, as compared to PC. Error bars show mean ±SEM. *p<0.05; ** p<0.01; *** p<0.001; ns = not significant

A)



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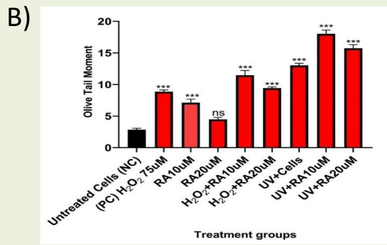
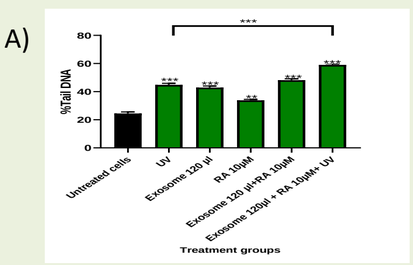


Figure 3 demonstrates the concentration-response of RA 10µM and 20µM and UV on CHL-1 cell line by %Tail DNA and OTM. The CHL-1 cells had a treatment with two concentrations of RA 10µM and RA 20µM. Results from the %Tail DNA and OTM showed a significant increase in DNA damage with RA 10µM compared to the untreated control. n=3 Error bars explain Mean±SEM. (*p<0.05); (** p<0.01); (*** p<0.001).

A)



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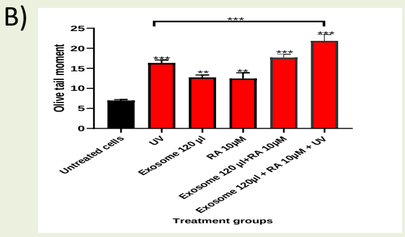

Figure 4. The effect of 10 µM RA and120µl exosomes, either alone or in combination with both on DNA damage in the presence of UVA+B in CHL-1 cells by analysing the % Tail DNA. The data were analysed by one-way ANOVA and Dunnett's post hoc test as mean ± SEM, n = 3 (* p <0.05; ** p <0.01; *** p <0.001; ns = not significant.

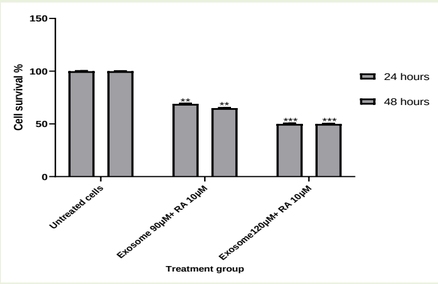

Figure 7 Two different combined treatments 9% (v/v) Exosomes + 10µM RA and 12% (v/v) Exosomes + 10µM RA were tested against the FM55 and CHL-1 cell lines. Measurements were done at 24 h and 48 h. All treatments reduced the Viability of cancer cells, with the most substantial effect seen for 12% (v/v) CBSC-derived Exosomes combined with 10 µM RA.

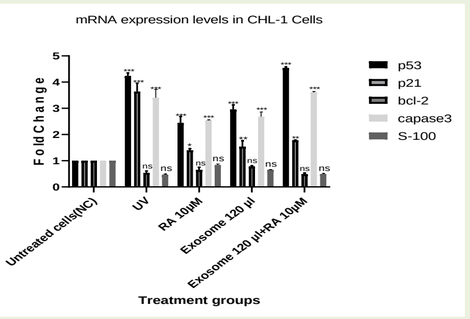

Figure 6 The effect of 10 µM RA and 120 µl exosomes alone or in combination in stressed UVA+B (1.2mW/cm2) CHL-1 MM cells on the gene expression level of *TP53*, *TP21*, *Bcl-2*, *S100* and *Caspase-3*. The treatment groups were compared against untreated cells (NC) and normalised against the internal housekeeping gene (β-actin). The significant upregulation of expression of *P53*, *P21* and *Capase-3* at mRNA level was observed with 10 µM RA and 120 µl exosomes alone and a more substantial effect of the same genes was observed in the combination treatment (10 µM RA + 120 µl exosomes). In contrast, *Bcl-2* expression was downregulated.

Conclusion

- Low-dose retinoic acid (RA) is a Practical Therapeutic Approach in MM
- High Optimum Doses of CBSC-Derived Exosomes in the Treatment of Melanoma Appears Promising
- Our study concludes that RA and exosomes Exhibit an anticancer effect in FM55 and CHL1 melanoma cell lines.
- The RA and CBSC- derived exosomes could be used as an anti-cancer and biological treatment to cure malignant melanoma.

References

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