Using nanoparticles to improve the therapeutic index of navitoclax for the treatment of ovarian cancer

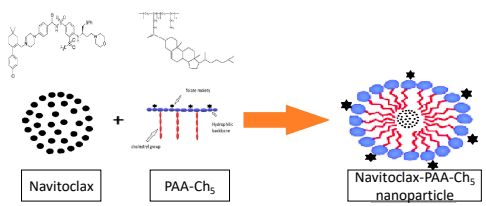
Khaled ALROSAN[1], Clare Hoskins[2], Alan Richardson[1]

[1]School of Pharmacy, Keele University

[2]Pure and applied chemistry, University of Strathclyde

Introduction

- Ovarian cancer (OC) has a high mortality rate as most patients are diagnosed at a late stage (1). It is considered the most lethal gynaecological cancer and the second most common type of genital cancer in female (2).
- It is the 5th most common cancer in women with about 7,000 new cases each year in United Kingdom (3). Furthermore, the 5-year survival rate for patients diagnosed with advanced ovarian cancer is very low, with around 45% and less than 30% in patients with late-stage disease(4).
- Its treatment is difficult, prolonged and complex. It is frequently characterized by recurrence after treatment, and repeated rounds of standard of care drugs such as carboplatin and paclitaxel show decreasing in benefits due to the development of drug resistance (5).
- The introduction of BH3 mimetics in the treatment of cancer is regarded as a fulfilment of a dream of many oncologists in the development of drugs that are capable of direct promoting apoptosis in cancer cells by antagonizing the Bcl-2 family of proteins (6) and resensitizing resistant cells to chemotherapy.
- The BH3 mimetic ABT-263 "Navitoclax", has been shown to induce cell death in lymphoma and leukaemia cell lines, in addition to tumour regression in xenograft studies of multiple myeloma and B-cell lymphoma (7). In addition, it was shown to increase the anti-cancer potency of carboplatin, and paclitaxel when used in combination (8)
- Dose-limiting thrombocytopenia was observed in patients treated with Navitoclax. This was likely to be due to the antagonism of the pro-survival function of Bcl-X_L in platelets (9). Thus, new strategies to target navitoclax toward ovarian cancer cells and to avoid accumulation in platelets may be beneficial. Formulation of navitoclax as nanoparticles is one such strategies that can be used to decrease navitoclax accumulation in platelets.



Aims

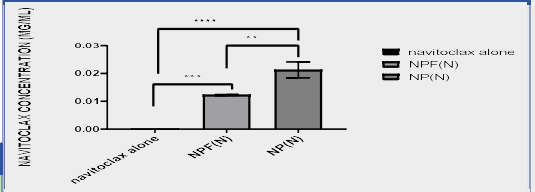
This research project aims to:

- Encapsulating navitoclax into Poly (allylamine)-cholesteryl (PAA- Ch_5) micelles and evaluate drug loading and release from the micelles.
- Compare the cytotoxicity of encapsulated navitoclax (navitoclax-PAA), free navitoclax and drug-free micelles towards ovarian cancer cells using several assays.
- Evaluate whether the synergy previously observed between free navitoclax and carboplatin is also observed with encapsulated navitoclax using several cytotoxicity assays.
- Evaluate whether combining folic acid with PAA-Ch_5 polymer improves the activity of the encapsulated navitoclax both alone and in combination with carboplatin

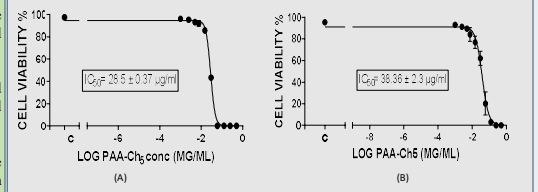
Methodology

1. Loading of navitoclax inside the PAA-Ch_5.
   - Nanoparticles of polyallylamine (PAA-Ch_5) were loaded with navitoclax. The amount of navitoclax incorporated into the nanoparticles, and the release of navitoclax from the nanoparticles, was measured by HPLC. The physical characteristics of the nanoparticles were assessed by photon correlation spectroscopy.
2. Measurement of cytotoxicity.
   - The cytotoxicity of empty nanoparticles, nanoparticles containing navitoclax and free navitoclax was measured in two ovarian cancer cell lines, OVCAR-8 and OVSAH using trypan blue assay, caspase 3/7 assay and PARP cleavage assays.
3. Loading of navitoclax inside nanoparticles modified with folate.
   - PAA-Ch_5 nanoparticles modified by addition of folate were also prepared. The same assays were used for the unmodified PAA-Ch_5 nanoparticles.
4. Measurement of cytotoxicity of the folate modified nanoparticles
   - The biological activity of the folate modified nanoparticles was characterized using the same assays as used for the unmodified nanoparticles.

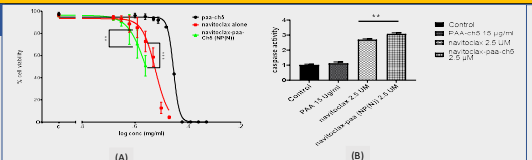
Results



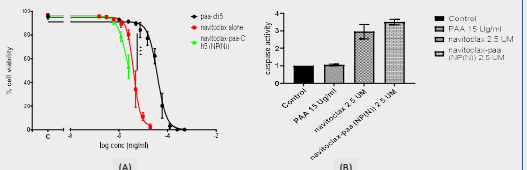
Improvement in navitoclax solubility when encapsulated in PAA-Ch_5 and the PAA-Ch_5-Fa. This figure represents the maximum concentration of navitoclax that has been encapsulated inside the PAA-Ch_5-fa core and inside the PAA-Ch_5 , this Concentration represents the solubility that has been achieved which is 62x and 100x better than the original solubility, respectively. (n=3 ± S.D)



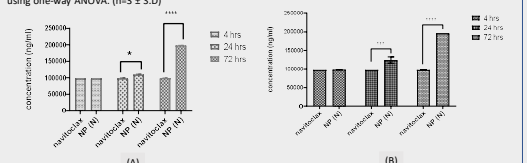
Identification of a non-toxic concentration of PAA-Ch_5 nanoparticles. This figure shows the cytotoxicity of PAA-ch5 without navitoclax against (A) OVCAR-8 and (B) OVSAHO using trypan blue assay. A nanoparticle concentration of 15 µg/ml was used in subsequent experiments because this concentration of the empty nanoparticles as non-toxic concentration (viability more than 80%) (n=3 ± S.D)



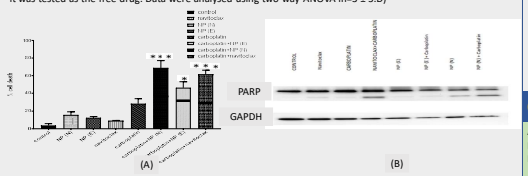
Cytotoxicity of navitoclax towards OVCAR-8 cells is enhanced by its incorporation in PAA-Ch_5 nanoparticles. This figure shows the cytotoxicity of both navitoclax-PAA-ch5 and navitoclax alone assessed with (A) trypan blue assay. . The data were analysed using two-way ANOVA. (n=3 ± S.D). (B) caspase 3/7 assay. The data were analysed using one-way ANOVA. (n=3 ± S.D)



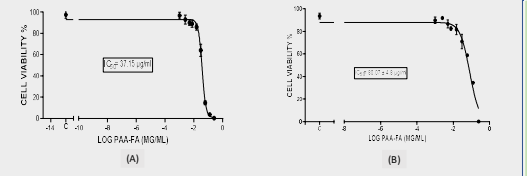
Cytotoxicity of navitoclax towards OVSAHO cells is enhanced by its incorporation in PAA-Ch_5 nanoparticles. This figure shows the cytotoxicity of both navitoclax-PAA-ch5 and navitoclax alone assessed with (A) trypan blue assay. . The data were analysed using two-way ANOVA. (n=3 ± S.D). (B) caspase 3/7 assay. The data were analysed using one-way ANOVA. (n=3 ± S.D)



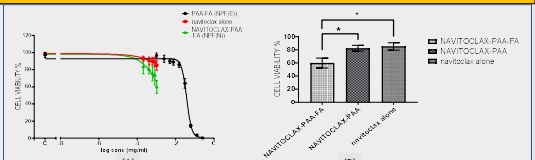
Uptake of navitoclax nanoparticles by ovarian cancer cells is augmented by encapsulation in PAA-Ch_5 nanoparticles. This figure shows uptake of navitoclax and navitoclax-paa-ch5 (NP(N)) by (A) OVCAR-8 and (B) OVSAHO. There was greater uptake of navitoclax by the cells when it was formulated in nanoparticles than when it was tested as the free drug. Data were analysed using two-way ANOVA in=3 ± S.D)



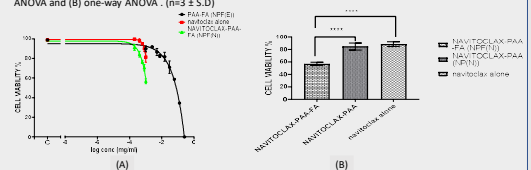
PAA-Ch_5 nanoparticles containing navitoclax are synergistic with carboplatin. The effect of the nanoparticles containing navitoclax was tested both alone and in combination with carboplatin using Ovcar-8 cells. The indicated cells were exposed to combinations of 13 µM carboplatin. 1 µM navitoclax-nanoparticles (NP(N)), 1 µM free navitoclax, or 6 µg/ml PAA without navitoclax (NP(E)) for 72h. (A) Cell death was determined by staining with trypan blue. The data (mean ± SD, n = 4) are expressed as a fraction of the number of cells alive. The expected effect of the combination assuming the drugs interacted additively is indicated as a solid horizontal line on the bar chart and was calculated using the Bliss independence criterion. There is a significant difference between the expected additive effect and the experimental results where indicated. Data were analysed using one-way ANOVA (B) Using western blot assay.



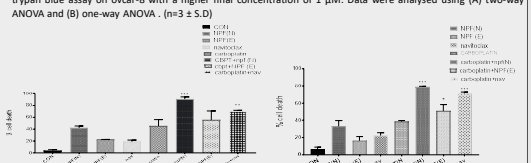
Identification of a non-toxic concentration of PAA-Ch_5 nanoparticles containing folate. This figure shows the cytotoxicity of PAA-ch5 without navitoclax against (A) OVCAR-8 and (B) OVSAHO using trypan blue assay. A nanoparticle concentration of 20 µg/ml was used in subsequent experiments because this concentration of the empty nanoparticles as non-toxic concentration (viability more than 80%) (n=3 ± S.D)



Cytotoxicity of navitoclax towards OVCAR-8 cells is enhanced by its incorporation in PAA-Ch_5-Fa nanoparticles .This figure represents (A) the Cytotoxicity of both navitoclax-PAA-ch5-FA (NPF(N)) and navitoclax alone and (B) the Cytotoxicity of both navitoclax-PAA-ch5-FA (NPF(N)), navitoclax-PAA-ch5 (NP (N)) and navitoclax alone using trypan blue assay on ovcar-8 with a higher final concentration of 1 µM. Data were analysed using (A) two-way ANOVA and (B) one-way ANOVA . (n=3 ± S.D)



Cytotoxicity of navitoclax towards OVSAHO cells is enhanced by its incorporation in PAA-Ch_5-Fa nanoparticles .This figure represents (A) the Cytotoxicity of both navitoclax-PAA-ch5-FA (NPF(N)) and navitoclax alone and (B) the Cytotoxicity of both navitoclax-PAA-ch5-FA (NPF(N)), navitoclax-PAA-ch5 (NP (N)) and navitoclax alone using trypan blue assay on ovcar-8 with a higher final concentration of 1 µM. Data were analysed using (A) two-way ANOVA and (B) one-way ANOVA . (n=3 ± S.D)



PAA-Ch_5-FA nanoparticles containing navitoclax are synergistic with carboplatin This Figure represents The effect of NPF(N) and navitoclax alone when given in combination with carboplatin on (A)ovcar-8 (B) OVSAHO. The indicated cell was exposed to 13 µM carboplatin. 1 µM navitoclax-paa (NPF(N)), 1 µM navitoclax, 20 µg/ml PAA-FA (NPF(E)) for 72h. Cell death was determined by microscopy after staining of the cells with trypan blue. The data (mean ± SD, n = 3) are expressed as a fraction of the number of live cells that were measured after exposure to with drug.. There is a significant difference between the expected additive effect and the experimental results where indicated. Data were analysed using one-way ANOVA

Conclusion

Adding of navitoclax to modified PAA-ch5 polymer with FA and unmodified polymer showed a successful Encapsulation and this was justified by the physical characteristics for the nanoparticles that were formed. Furthermore, this encapsulation led to a an increment in the water solubility of navitoclax which represents a revolution since navitoclax is administered in oil based formulation.

Whereas nanoparticles have a special characteristic to penetrate inside the tumour tissue through EPRE, and also with active targeting. The encapsulated navitoclax showed a double toxicity when compared with navitoclax alone and also a higher synergistic effect when given with carboplatin.

Accordingly, this means an improvement in the therapeutic Effect of navitoclax and possibility of using a lesser concentration with the same effect and less side effects. However, this needs to be further experimented *in vivo* studies.

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