Control of cell death levels using TH9402-based PDT treatment on fresh PBMC, and potential application to patients with cGVHD.

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1. Development of PDT conditions

1.1. Levels of cell death directly correlates with TH9402 concentration and light intensity

We analyzed the correlation between the concentration of TH9402 and the energy of illumination with the level of PDT-induced apoptosis, using PBMC isolated from healthy volunteers. TH9402 was directly proportional to the concentration of TH9402 (Fig 1A). Cells were illuminated after 90min of coloration. The levels of early apoptosis measured by AnnexinV/PI staining at 3 hours post-PDT were proportional to both the TH9402 concentration and energy of illumination (Fig 1B). The correlation of late apoptosis with the energy of illumination was attenuated in cells fixed 3 days post-PDT, whereas the levels of apoptosis were still directly proportional to the dose of TH9402 (Fig 1C).

1.2. Flexibility Study

The data obtained from samples isolated from 44 subjects is in agreement with the number of treatments done at each condition, which is the most predictable results that could be obtained with different dose of TH9402. Inclucluing cells with 0.33 µM of drug gave 35±13% of apoptosis, whereas cells when treated with 0.66 µM of TH9402, respectively (Fig 1C). TH9402 was stimulated with TH9402 alone were tested no effects.

1.3. TH9402-based PDT shows low variability in PBMC isolated from healthy donors

The t-test and one-way ANOVA analysis on drug uptake and apoptosis were calculated using Prism software from healthy volunteers to PDT in vitro for each condition. The cellular uptake of TH9402 was significantly correlated with the energy of illumination (Fig 1A, 1B).

1.4. TH9402-PDT coloration time appears robust in healthy donors

The efficacy of PDT to induce apoptosis was tested on PBMC isolated from 3 healthy volunteers incubated with TH9402 for 45, 60 or 90 minutes. The drug uptake was monitored by FACS during coloration (Fig 1A) and the levels of apoptosis were measured by AnnexinV/PI staining 3 hours post-PDT (Fig 1B) and TUNEL assay 3 days post-PDT (Fig 1C). Final levels of apoptosis were on average 10% and 20% for cells incubated with 0.33 and 0.66µM of TH9402, respectively (Fig 1C). Interestingly, incubating cells for 45, 60 and 90 minutes with the drug had no significant impact on the levels of early (Fig 1B) and late (Fig 1C) apoptosis.

2. Application to Cells Isolated from cGVHD Patients

The results obtained from the analysis of apoptotic levels of cells killed 3 days post-PDT revealed similar sensitivities to the treatment between cells isolated from healthy volunteers (38±9%) and cGVHD patients (28±16%) (Fig 1F). When treated with 0.33µM of TH9402, cells isolated from cGVHD patients or from healthy donors showed no significant difference with 38±9% and 28±16% of apoptosis 3 days post-PDT, respectively. Similarly, there was also no significant difference in cell death between these cells treated with 1.32µM of TH9402, with 73±13% and 74±11% of apoptosis, respectively.

3. Mechanistic Studies

3.1. PDT induces apoptotic cell death

The effect of the PDT-treatment on the Jurkat T-cell line was compared to the effect of the well-known apoptosis inducing compound (4-46-M). The mitochondrial depolarization was monitored in cells treated with AnnexinV/PI staining as compared with untreated cells, which was treated with 73±13% and 74±11% of apoptosis, respectively.

3.2. Proliferating Jurkat cells are more sensitive to PDT than resting PBMC

In fact, when proliferating Jurkat cells and resting PBMC were treated in parallel, 79% of Jurkat T-cells were apoptotic 2 days post-treatment with 6µM of Z-VAD-fmk. The caspase-inhibitor induced a significant decrease of apoptosis levels in proliferating Jurkat cells to 35±13% and 36±13% respectively (Fig 2A, 2B).

3.3. – PDT-induced apoptosis may be either caspase-dependent or caspase-independent

In parallel to the treatments described in 3.2, cells were treated in the absence of Z-VAD-fmk. The caspase-inhibitor induced a significant decrease of apoptosis in both Jurkat cells and in resting PBMC treated with 0.33µM of TH9402, whereas 48% and 58% of PBMC were apoptotic when treated with 0.33µM, 0.66µM and 1.32µM of TH9402, respectively (Fig 3A).

4. – Activated PBMC are more sensitive to PDT than resting cells

PBMC isolated from 3 donors were stimulated with PHA for 3 days prior to PDT (Fig 3A). In PHA-stimulated PBMC, activated lymphocytes incorporated significantly more TH9402 than non-activated lymphocytes (Fig 3B). The results obtained by TUNEL assay from stimulated and non-stimulated cells showed a strong linear correlation between apoptotic levels and concentration of TH9402, and in PHA-stimulated cells were significantly more sensitive than non-stimulated cells to PDT (Fig 3C).

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