L-DOS47 – a novel lung adenocarcinoma specific immuno-conjugate therapeutic

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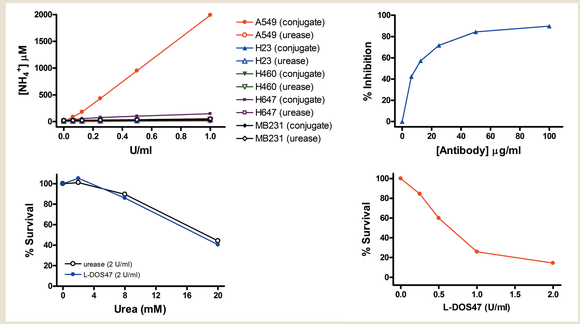
Summary

It has been observed that solid human tumours produce a local microenvironment that is more acidic than normal tissue (1,2). This phenomenon can impact the efficacy of certain chemotherapeutics by altering their partitioning coefficient between extracellular and intracellular compartments due to charge alternations in the drug (3,4). Also, it is shown that this metabolic condition can induce metastasis and confer a growth advantage to certain cancers (1). An immuno-conjugate cancer therapeutic that is designed to exploit the acidic tumour extracellular environment has been developed. The molecule, L-DOS47, is composed of a lung adenocarcinoma specific single domain antibody (5) and a urease enzyme. The antibody serves as a targeting agent to deliver the enzyme to the affected sites while the urease enzyme converts urea, an abundant metabolite, into ammonia and generates a local pH increase. The combined effect of ammonia toxicity and pH increase is cytotoxic to cancer cells in culture and in xenograft models (6). L-DOS47 binds specifically to human lung adenocarcinoma as shown by tissue specific staining. Imaging studies using A549 xenografts and intravenously-given labelled L-DOS47 showed that the drug molecule preferentially accumulated at the tumour site. L-DOS47 persisted at the site well over 72 hours, consistent with a strong binding antibody. In vitro experiments showed that L-DOS47 potentiated the cytotoxic effect of a number of chemotherapeutics. In some instances, less than one tenth of the normal drug dose was required to achieve the desired cell growth inhibition effect. In summary, L-DOS47 is a novel immuno-conjugate that preferentially targets lung tumours and generates cytotoxicity directly through its enzymatic function or in combination with weakly basic chemotherapeutics through a potentiated pH effect.

Study Outline

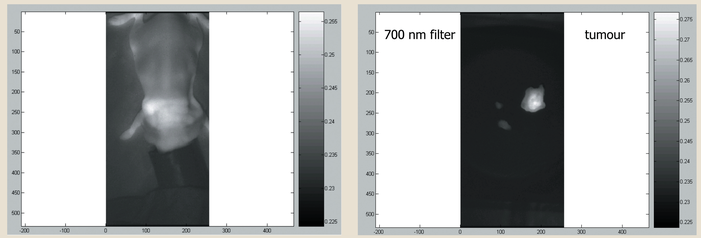
1. Effect of urease on A549 and MDA-MB231 cell
2. Effect of L-DOS47 on tumour cell lines
3. Ability of L-DOS47 to potentiate weakly basic drug
4. Fluorescent Imaging of L-DOS47 in A549 xenografts
5. L-DOS47 binding to human lung tumor tissues

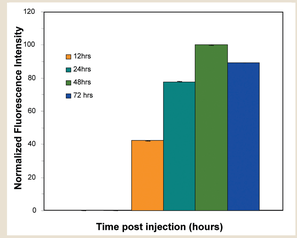
Effect of L-DOS47 on tumour cells



Five cell lines (A549, H23, H460, H647, and MDA-MB-231) were seeded in 96-well culture plates to test for the binding specificity of L-DOS47(top left). L-DOS47 binds specifically and significantly only to A549 cell – a human lung adenocarcinoma line. The immuno-conjugate also generates ammonia in the presence of urea. Specificity of the binding is confirmed by competitive displacement with the antibody alone in a dose dependent manner (top right). In a solution assay (without washing), L-DOS47 and unconjugated urease have the same ability to kill A549 (bottom left). However, when rinsing is introduced after enzyme alone or L-DOS47 addition and prior to the addition of urea, only L-DOS47 is retained on the cell and remains active (data not shown). Similar to urease, L-DOS47 is able to kill A549 cells in a dose dependent manner (bottom right).

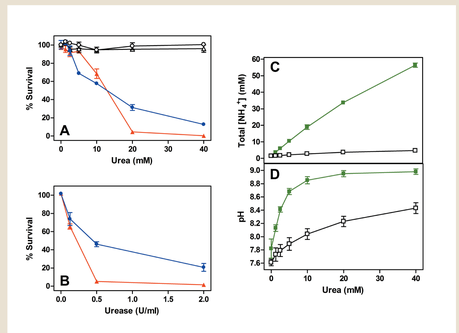
Fluorescent Imaging of L-DOS47 in A549 xenografts





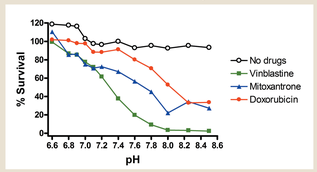
Inactivated L-DOS47 labelled with Cy5.5 was injected in SCID mice bearing A549 xenograft. A single injection of 100ug of Cy5.5-L-DOS47 was made through the tail vein. Mice were anaesthetised at specific intervals and full body fluorescence images were taken. Animals were sacrificed at time intervals. The tumour and major organs were harvested for imaging. Relative signal intensities were used to measure bio-distribution, half life and accumulation. A representative picture of a tumour bearing mouse is presented on the top left panel. The image was taken at 72h post injection of Cy5.5-L-DOS47 – note implanted tumour on right shoulder. Tumour from the same animal was harvested and photographed separately (top right). Summary result showing signal accumulation in tumour (ex vivo) as a function of time is presented in the bottom panel. Detectable Cy5.5- L-DOS47 began at 24 hours and persisted beyond 72 hours.

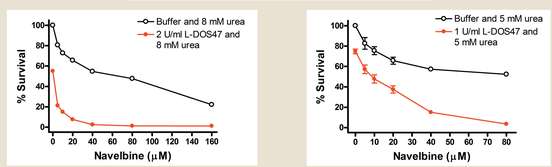
Effect of urease on A549 and MDA-MB231 cells



Urease induced cytotoxicity on A549 and MDA-MB-231 cells and augmented the pH and ammonium content of the culture medium. A, A549 (▲) and MDA-MB-231 (●) cells were treated with 2 U/ml of urease in the presence of 0 – 40 mM of urea and incubated at 37°C for 2 hours. Viability of the treated cells began to drop as the urea level increased. Urea alone had no effects on A549 (Δ) and MDA-MB-231 (○) controls. B, dose-response curve of urease on the viability of A549 and MDA-MB-231 cells. The tumour cells were subjected to urease treatment in the presence of 20 mM urea for 2 hours. A549 cells (▲) were more susceptible to urease than MDA-MB-231 cells (●). *C* and *D*, total ammonium and pH measured in pooled incubation buffer collected from *A*. Hydrolysis of urea by urease (■) caused an increase in ammonium content (*C*) and pH (*D*) as compared to the controls (□). Values are means ± S.D. of 4 replicates from 3 experiments.

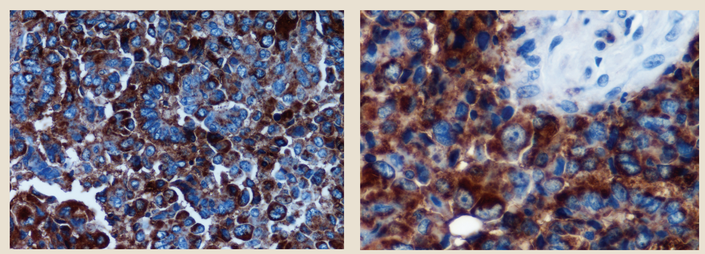
L-DOS47 potentiates weakly basic drugs in A549





The cytotoxic effect of weakly basic drugs is directly related to the solution pH (top panel). At an acidic tumour pH (<6.8), the effectiveness of these drugs are significantly reduced. L-DOS47 can dramatically raise the effectiveness of these drugs (e.g. navelbine, bottom panels). This synergistic effect is directly related to its enzymatic properties of generating ammonia from urea and raising solution pH. Depending on the dosages and available urea, a 2 – 10 fold drug effect enhancement can be observed.

L-DOS47 binding to human lung tumour tissues



Human lung adenocarcinoma tissue biopsies were sectioned and prepared into slides. After blocking the slides in serum at 4°C overnight, the slides were incubated with L-DOS47 (20 U/ml) at 37°C for 1.5 hours. After washing, the slides were incubated with mouse anti-urease antibody (1/1500x) at 37°C for 1 hour, followed by diluted biotinylated secondary antibody solution for 30 min. After incubation, the slides were treated with Vectastain Elite ABC Reagent for 30 min. Colour was developed in fresh DAB solution at RT for 2 minutes. L-DOS47 stains are brown in colour with blue colour counter stain.

References

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