Polyethylene glycol glucose conjugate: an enabling excipient to block overexpressed glucose transporters on malignant cells.

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Editorial

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There are as many mutated or gone awry potential biological targets in hyper-proliferative cells as there are steps in the biological processes of cellular respiration, metabolism, apoptosis and glycolysis. This heterogeneity is the single greatest obstacle to cancer therapy. Even the Warburg effect, i.e. that of hypoxic cancer cells primarily resorting to glycolysis to fulfill their energy needs due to impaired oxidative phosphorylation, does not seem to be true of many cancers. However, a commonality between many cancers is that they have increased rates of glucose uptake that is, in turn, achieved by overexpression of cell membrane localized uniporters called glucose transporters or GLUT. A 'near universal' approach to kill cancer cells lies in depriving them of their metabolic fuel, i.e. the preferential blockage of GLUT on malignant cells while sparing GLUT on normal cells. How might this be achieved?

As a first approximation, if glucose molecules could be conjugated to polyethylene glycol (PEG-Glu) at an end-to-end span distance lesser than the mean distance between two glucose transporters in normal cells, it could theoretically be possible to preferentially disable GLUT receptors on malignant cells on an equimolar excipient basis. An internet search obtained one hit that espoused substantially the same idea in the US patent 20,110,243,851 A1 entitled "Glucose-PEG conjugates for reducing glucose transport into a cell" (1). While proof of concept was demonstrated in this patent application, the inventors did not suggest any method for sparing GLUT on normal cells while only blocking those on malignant hyper-proliferative cells. They also envisaged the use of this molecule as a drug rather than as an excipient.

Overexpression of GLUT transporters in the cell membrane of malignant cells must necessarily decrease the mean distance between two transporter-receptors. If the end-to-end distance between the glucose conjugated sites of the PEG molecule approximates this decreased distance, then the glucose moieties in the PEG-glucose conjugate should

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preferentially bind to malignant cells. Differential glucose deprivation of malignant cells is expected to cause necrosis or apoptosis of those cells depending on the functional state of the mitochondria.

Using the following general approximations:

Weight of one cell: 1 ng
Expression of GLUT transporters in a normally respiring cell: 15 [pmol/mg]
Diameter of an average cell: 50 μm.
Mean molecular weight of GLUT isoforms: 45 kDa
Mean cross-sectional pore area of trans-membrane GLUT transporter: 5 pm

and, assuming that the GLUT transporters are evenly distributed across the cell membrane, the calculated surface area of a cell works out to be 7850 X 10^6 nm^2 and there are 1.15 transporters per 1000 nm^2 cell surface. Therefore, the mean distance between the GLUT transporters on the cell membrane is 31 nm. Since the carbon-carbon bond length is 0.154 nm, there would need to be 200 carbon atoms between the two glucose residues conjugated to the PEG molecule. A 2.4 kDa PEG conjugated at both ends with glucose molecules should therefore bind to the GLUT transporters on non-transformed cells. It can be assumed that a PEG molecule with a molecular weight significantly lesser than 2 kDa will only be able to block one GLUT receptor per molecule.

Assuming a two-fold, or greater, expression of GLUT transporters on transformed or hyper-proliferative cells, the mean GLUT distance will be halved to 15 nm or less. Since the Michaelis constant for GLUT-1 transporters for glucose is 16.9 to 26.2 mM, and the GLUT-1 isoform is the one reported to be the most over expressed on malignant cell lines, it is reasonable to conclude that the binding enthalpy of glucose and GLUT is not significantly greater than the hydrogen bonding enthalpy between the PEG-glucose conjugate and aqueous blood such that equimolar concentrations of the conjugate will accumulate on both malignant cells over-expressing the GLUT transporter as well as on non-malignant cells. Therefore, as a first approximation, it can be reasonably concluded that a PEG with a molecular weight of 1000 Da or less, that is conjugated at both ends (as well as on the intermediate carbons) with glucose moieties will disable proportionally more GLUT receptors per mole on malignant cells than on normal cells at non-saturated ligand concentrations. Note that the model assumes rigid C-C bonds incapable of rotation about the bond axis and that stearic effects are neglected. No doubt, much better approximations will be possible with molecular docking and binding software simulations although the driving principle of the hypothesis is expected to remain unchanged.

Since the glucose is conjugated to the PEG through its aldehyde containing C-1 carbon, it is no longer a reducing sugar and cannot degrade the API through reactions characteristic of such sugars (see Figure 1 for a proposed structure of the molecule). The molecule is expected to behave as a non-crystalline entity when frozen thereby affording protection to protein APIs' during lyophilization *via* the water-replacement hypothesis. Aqueous solutions of PEG-Glu are expected to exhibit a lower dielectric constant than water, thereby allowing a greater amount of API dissolution with a lesser amount of surfactants. The molecule thus appears to be no less inferior than existing excipients in its

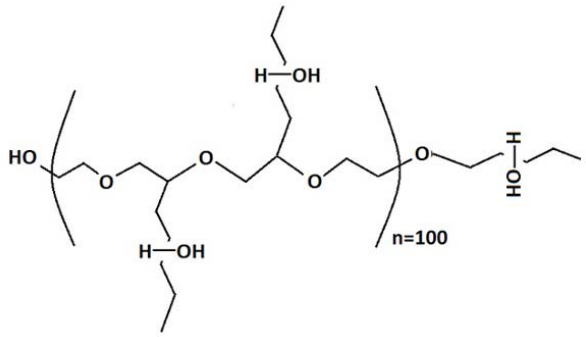

Figure 1 Proposed PEG-Glu Structure

solubilizing and stabilizing properties and could thus be envisaged as an excipient in lyophilized (small molecule or protein) formulations or as an infusion medium *in leu* of D5W or saline for intravenous infusion or bolus administration of antineoplastic drugs.

The current state of excipient innovation is essentially limited by what combinations and permutations of molecules can be siphoned off from the food industry. While the example of PEG-Glu structurally fits into that category, this molecule as described in this editorial has been intentionally designed, rather than accidentally found, to be *in vivo* enabling. There are many excipients that have been serendipitously discovered to possess bioactive target engagement capability but were not explicitly so designed for that purpose. As just one example, the broad range of surfactants used in pharmaceutical dosage forms have been found to possess varying magnitudes of P-glycoprotein modulatory function that is now recognized as a primary effector in preventing multi drug resistance (MDR). Therefore, evidence of pharmacological *in-vivo* target engagement (whether deliberately built into the structure or found after years of use), in and of itself, is not sufficient to deny an 'excipient' designation to molecules so long as they perform their usual (obligatory) functions of filler, binder, solubilizer etc. An API, tranexamic acid, has been used as an excipient in Reteplase® as a solubilizing agent for the non-glycosylated protein molecule. Precedence notwithstanding, many pharmaceutical excipients and even food ingredients possess *de-novo* pharmacological activity (and hence, by definition, modulate cellular receptors and/or targets), as indeed, does glucose itself via its GLUT pharmacological target.

Although polyethylene glycols are not absorbed from the gut, could decreasing their molecular weight by orders of magnitude (< 5 carbon backbone) enable oral bioavailability while preserving their GLUT blocking functionality? When ligated with sucrose (PEG-Suc) could they be absorbed from the gut? In another admittedly speculative twist to this intriguing story; would it then make sense from a public health viewpoint to add such low molecular weight PEG-Suc to sugar for mass consumption? *In vivo* enzymatic hydrolysis of sucrose to glucose would leave PEG-Glu that would function as a proliferative cell inhibitor. Could PEG-Gly be added to isotonic D5W or saline or, even be substituted for the latter, especially for infusing cancer patients?

The pharmaceutical industry should examine unfulfilled *in vivo* needs, such as that described above, that could be met by excipients, either alone or in synergistic combination with active ingredients. This will enable innovative a' priori design of new excipient molecules to provide value added propositions to the pharmaceutical industry. Explicit recognition by regulatory agencies that *in vivo* receptor target engagement is not necessarily alien to excipient property, design or function will go a long way toward expanding excipient innovation and scope. The intent of such a paradigm is not to somehow deliberately endow excipients with pharmacological properties, that most (beknownst or unbeknownst) already possess , but to take these cogent issues of excipient definition, properties and function into account when formulating regulatory policy for pharmaceutical excipients.

REFERENCES

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