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The evaluation of a novel class of non-toxic absorption enhancer

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The delivery of peptide and protein drugs is problematic and the subject of intense research. Absorption Enhancer's (AE's) may be useful for delivery of these types of molecules, but their usage is limited due to toxic effects at the level of the cell membrane, (De Boer et al 1990). This report describes the evaluation of a novel class of peptide-based AE, that facilitates a reversible, dynamic absorption enhancement. A total of 12 have been synthesised so far, designated AE1-AE12, and are currently the subject of a patent application in view of the promising results obtained so far.

A number of studies have been conducted to evaluate the properties of these AE's. Liposomal dye release studies have shown that this class of AE can perturb the bilayer and afford the release of a fluorescent dye. Some of these AE's allow complete dye release in matter of minutes (AE7 and 12), whereas others allow only 20% of the entrapped dye to be released after 1 hour (AE's 8 and 10). Haemolysis studies were performed to assess the toxicity of these molecules. AE's 7 and 12 caused 100% haemolysis after a 30 minute incubation period with the cells, whereas AE's 8 and 10 caused less than 5% haemolysis under similar conditions.

These preliminary screens identified AE8 as a suitable candidate for further study in a Caco-2 model. A radiolabelled octapeptide was synthesised using a conventional solid phase methodology, (Atherton and Sheppard 1989), and used in transport experiments. At a concentration of 0.25mM, AE8 was seen to increase the rate of flux of an octapeptide threefold relative to a negative control and was half as effective as a 0.4mM SDS solution over a 4 hour period (see Figure 1). This flux was observed to occur most rapidly in the first hour after which the rate returned to control levels. The toxicity of AE8 in the Caco-2 model was assessed by the 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium (MTT) assay, lactate dehydrogenase assay and by trans-epithelial electrical resistance measurements. All three methods have established that toxicity is not apparent when AE8 is used at concentrations of up to 0.5mM.

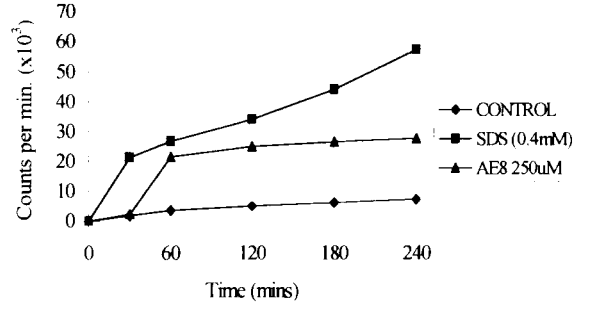

Figure 1:- the transport of a ^{14}C labeled octapeptide across a Caco-2 monolayer. (n=6)

We propose to elucidate the precise mechanism of absorption enhancement by using a confocal laser microscopy technique, (Sakai et al 1997), in conjunction with the fluorescently labeled octapeptide. This will allow us to visualise whether the activity of AE8 is mediated by a para- or transcellular modification.

In conclusion, we have prepared a novel range of peptide based AE's. Studies in Caco-2 cells have demonstrated that a transient, non-toxic absorption enhancement is possible. These molecules may have utility in the delivery of a broad range of pharmaceuticals including peptides and proteins.

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