

Nevertheless, the maximum tolerated dose was lower than that of the passively targeted HPMA-Dox (PK1). There was an accumulation of 15-20 % of the total dose in the liver as opposed to a general body distribution with PK1 (Seymour et al., 2002). The source of toxicity observed in PK2 over PK1 was not clear. Table 1.1 gave an overview of the current research on receptor targeting for drug delivery.

The antibody (anti-CD33)-calicheamicin (Mylotarg<sup>®</sup>) showed no cardiac or cerebellar toxicity, but grade 4 neutropenia and thrombocytopenia was observed (Sievers et al., 2001). It was however predicted that this type of toxicity may occur due to the targeting of CD33 which is also present on normal maturing haematopoietic progenitor cells (Sievers et al., 2001). Calicheamicin, the active component of Mylotarg<sup>®</sup>, does not appear to be used as a single drug entity. Therefore it is not possible to make a comparison to the single drug efficacy. From these studies it can be seen that targeting has a role to play in the arsenal against cancer but more specific targets should be sought to improve therapy.

A reduction of the side-effects of a gene therapy cannot be truly considered as this is a new field. The therapeutic gene could be engineered in such a way that no toxicity is expected and it may be the vector rather than the gene/protein which is toxic. In non-viral gene therapy targeting is used to overcome the cell membrane barrier and increase uptake, through receptor mediated endocytosis (RME), of the polyplex. This is addressed in Section 1.5.3.

### ***1.3.1 Passive Targeting of Drugs and/or Carriers***

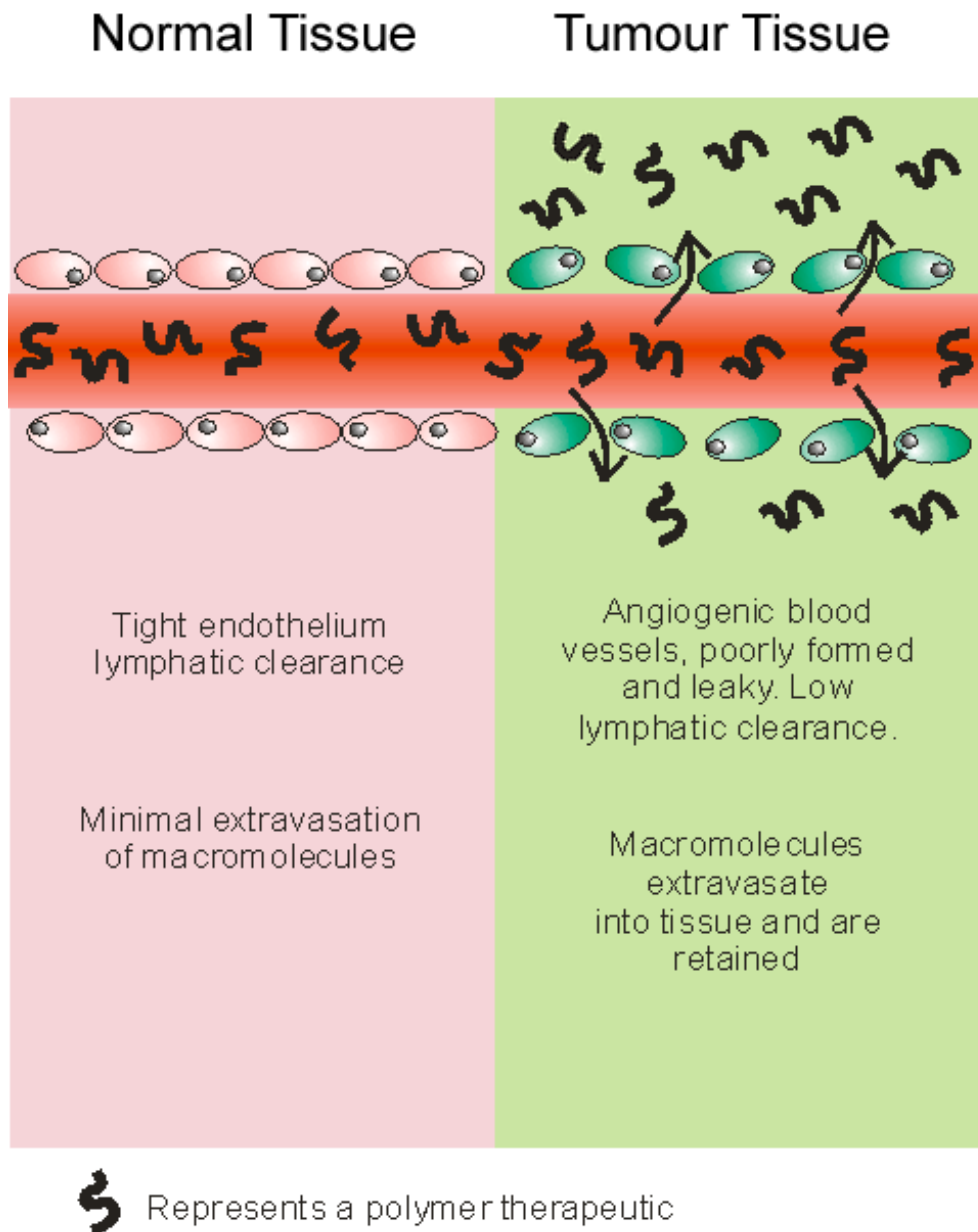
It was reported in 1984 (Maeda et al., 1984) that macromolecules can accumulate selectively in tumour tissues. Later it was observed that the polymer-protein conjugate styrene-maleic acid neocarzinostatin (SMANCS) selectively accumulated in mouse solid tumours (Matsumura et al., 1987). To explain this phenomenon Maeda and Matsumura introduced the term “enhanced permeability and retention (EPR) effect” (Matsumura et al., 1987). Neovascular tissue angiogenically formed by the tumour is disorganised, meaning macromolecules can extravasate into the interstitial space in a tumour (Dvorak et al., 1988). Neovascular tissue is formed by the tumour due to its increased nutrient requirements (Folkman & Shing, 1992, Satchi-Fainaro et al., 2004). The epithelium of these new blood vessels is disordered and therefore more permeable than normal vasculature (Maeda et al., 2000). Although now accepted, the EPR effect

was originally opposed as there is high intratumoural pressure which, it was thought, would prevent the extravasation of macromolecules. Passive targeting using the EPR effect has already been utilised in the clinical treatment and imaging of solid tumours and has wide applicability (Maeda et al., 2000). Liposomes, polymer-drug conjugates and nanoparticles all target tumours via the EPR effect and all polymer-drug conjugates in clinical development use the EPR effect for tumour specific targeting (Duncan, 2003). Nanoparticles consisting of polymeric materials and therapeutic(s) can be small enough to extravasate due to the enhanced permeability of neovascular tissue yet large enough to both be retained in the tumour due to the reduced lymphatic drainage, and not to extravasate in normal tissue (Brigger et al., 2002, Maeda et al., 2001, Satchi-Fainaro et al., 2004) (Fig. 1.4).

Polymeric colloids were found to accumulate in tumour xenografts in mice (Nakanishi et al., 2001). They studied a polymeric doxorubicin containing micelle formulation (NK911) consisting of poly(ethylene glycol) (PEG)-coated polyaspartic acid conjugated to doxorubicin which has an average size of 41.9 nm and was found to accumulate in tumours in mice 3.4-fold over free doxorubicin (Nakanishi et al., 2001).

Glycol-chitosan doxorubicin nanoparticles of up to 300 nm have been found to accumulate in tumour tissue in rats (Son et al., 2003). Although the actual cut off size value for extravasation in tumours due to EPR may be as large as 500 nm, it could vary in different tumours (Torchilin, 2000).

Polyplexes are formed through the interaction of negatively charged nucleic acids with positively charged polymers (Leclercq et al., 2003). Polyplex size is controlled by the concentration and type of polyelectrolyte used (Ogris & Wagner, 2002b) they generally have < 200 nm sizes (Koping-Hoggard et al., 2001, Reineke & Davis, 2003b, Reineke & Davis, 2003a). It is envisaged that polyplexes will accumulate in tumours due to the EPR effect (Ogris & Wagner, 2002b). This accumulation was reported in tumour bearing mice treated with poly-lysine dendrimer complexes when compared to accumulation with a liposome formulation (Kawano et al., 2004). It has also been found that at the site of the tumour, LTT enhances cell binding and uptake. LTT is the next idea considered.



**Figure 1.4 - Schematic showing the EPR effect**

In normal tissue the macromolecules/nanoparticles cannot escape the vascular endothelium. In contrast cancer tissue angiogenic neovasculature is more permeable allowing them to extravasate into the interstitial space. Lymphatic drainage of tumour tissues is impaired and macromolecules/nanoparticles are retained (Adapted from Duncan, 2003).