Literature review

2. LITERATURE REVIEW

2.1 Drug resistance associated with cancer chemotherapy

Therapeutic anticancer drugs must reach tumors by overcoming problems such as drug resistance at the tumour level due to physiological barriers (non-cellular mechanism) and drug resistance at the cellular level (cellular mechanism). In addition, they must successfully have the following attributes: distribution, biotransformation and clearance of anticancer drugs in the body. There are different mechanisms by which a tumour can be resistant to any therapeutic drug. This resistance is the cause of frequent failure in chemotherapy treatment with any chemotherapeutic drug or due to inherited resistance. The mechanisms of tumour resistance can be classified as non-cellular and cellular drug resistance mechanisms.

Non-cellular drug resistance mechanisms could be due to poorly vascularized tumour regions, which can effectively reduce drug access to the tumour and thus protect it from the cytotoxicity of the drug. The acidic environment in tumours can also confer a resistance mechanism against basic drugs. These compounds would be ionized, preventing their internalization across the cellular membrane.

Cellular drug resistance mechanisms compromise altered activity of specific enzyme systems, altered apoptosis regulation, or transport based mechanisms, like P-glycoprotein efflux system, responsible for the multidrug resistance (MDR), or the multidrug resistance associated protein (MRP).

Another problem faced by the use of anticancer drugs is their toxicity to both, tumour and normal cells, resulting in a limited efficacy of chemotherapy due to significant side effects. Furthermore, until recently, frequently repeated doses of pharmaceutically active agents were required for patient treatment to maintain desired drug levels and thereby also prolonged the significant side effects of these drugs. All of the above mentioned facts favoured the introduction of controlled drug release delivery formulations, such as nanoparticles, liposomes, microspheres, etc. Controlled-release technology has attracted much attention since it is possible to overcome such non-

cellular and cellular based mechanisms of resistance. Moreover, it is possible to increase the selectivity of drugs to cancers cells reducing their toxicity towards normal tissues by means of these formulations

2.2 Multidrug resistance

One of the major drug resistance mechanisms that act as a limiting factor in treatment of cancer is development of multidrug resistance (MDR). It is defined as the resistance of the tumor cell to a broad spectrum of structurally and mechanistically diverse antitumor agents (Vyas and Khar, 2002). Tumor cells carrying MDR phenotype are often associated with over expression of some of the drug efflux pumps, known as P-glycoprotein (Pgp) pumps and multidrug resistance associated protein (MRP) pumps (Kartener et al., 1985). It prevents intracellular accumulation of many anticancer agents (that are its substrates) and hence causes a reduction in their cytotoxic activity mainly increasing cellular efflux of positively charged amphipathic drugs in an ATP-dependent manner (Figure 2.1).
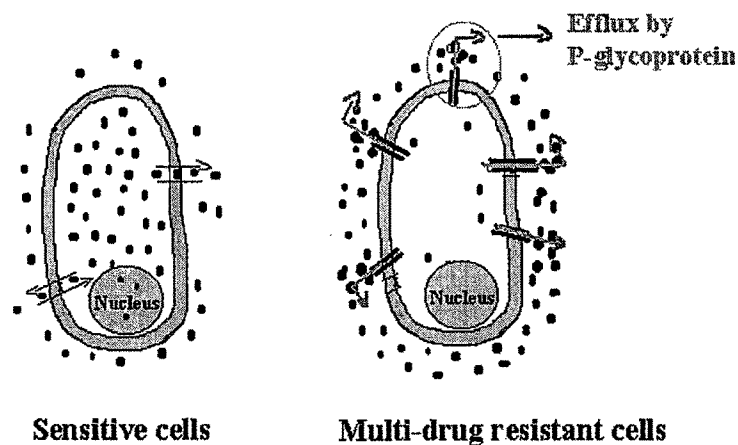

Sensitive cells   **Multi-drug resistant cells**

Figure 2. 1: Drug efflux phenomenon in multidrug resistant cells

2.2.1 P-glycoprotein

Pgp is a 170kDa membrane transporter of the ABC (ATP binding cassette) superfamily that was described initially in the broad field of cancer research (Beidler and Reihm, 1970; Ling and Thompson., 1974). ABC transporters share a common domain organization and considerable amino acid sequence identity, implying a common architecture and evolutionary origin. Pgp is the product of the ABCB1 gene (mdr1 and

mdr1b in rodents, and MDR1 in humans), which can be phosphorylated and localized in the plasma membrane as glycosylated transmembrane protein. Human Pgp (Figure 2.2) a membrane drug transporter is composed of 1280 amino acids. Pgps are N-glycosylated on the 1st extracellular loop in three different locations (Schinkel., 1999). This transmembrane protein has been thought to be organized into 12 transmembrane segments forming 2 hydrophobic domains each consisting of 6 transmembrane segments. There are also two ATP binding sites in the cytoplasmic area (Endicott and Ling, 1989; Gottesman and Pastan., 1993; Loo and Clarke., 1999; Ronison, 1992). This kind of arrangement of domains is a characteristic of the ATP binding cassette transporters (ABC transporters) of which Pgp is a member. ATP hydrolysis provides the energy for its active drug transport, which can occur against a steep concentration gradient.
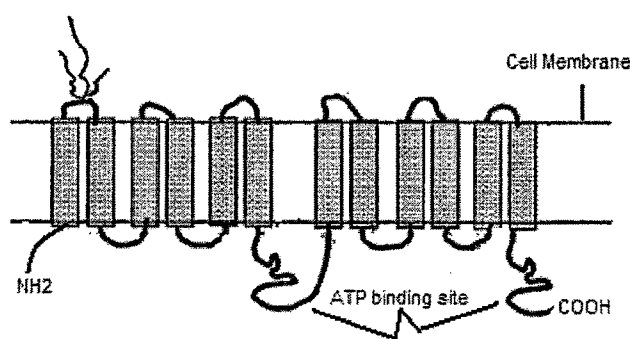

Figure 2. 2: Structure of P-glycoproteins

The genes encoding Pgp in humans are MDR1 and MDR3 genes. Latter is also known as MDR2 gene (Callen et al., 1987; Chin et al., 1989) whereas in rodents three genes have been found to encode the Pgps namely mdr1a, mdr1b and mdr2 (Ueda et al., 1986; Gros et al., 1987). In humans the MDR1 gene confers multidrug resistance while in rodents mdr1a and mdr1b genes confers resistance whereas MDR2 and mdr2 genes in humans and rodents respectively have no role to play in multidrug resistance.

Infact, they are believed to have been involved in transporting phosphatidyl choline across the canalicular membrane of hepatocytes (Smit et al., 1993; Reutz and Gros., 1994; Borst et al., 2000a). Cholesterol also interacts with the substrate binding site of Pgp suggesting that cholesterol is transported by Pgp (Wang et al., 2000). On the

contrary, MDR3 gene is shown to bind to some of MDR1 Pgp substrates and influence their transport across the cell but inefficiently (Smit et al., 2000). Also in recent times Pgp have been shown to play a role in cell differentiation as was demonstrated in murine bone marrow stem cells transfected with the MDR1 gene (Bunting et al., 2000) and regulation of cell death by Pgp (Johnstone et al., 2000). From the above results it can be assumed that Pgp could be a key player in the lipid trafficking in the important membrane microdomains involved in several crucial pathways in cell proliferation, signal transduction and transcytosis.

Apart from being located in the tumor cells, MDR1 Pgp is also located in many human tissues. The most prominent tissues among them are the apical membrane of intestinal epithelial cells, the biliary canalicular membrane of hepatocytes and luminal membrane of proximal tubular epithelial cells of kidney (Theibeut et al., 1987; CordonCardo et al., 1989; Jette et al., 1993). Good amount of Pgp is also found in the adrenal glands (of mice and humans and not in rats) and also in the endometrium of pregnant uterus and also in capillary endothelial cells in the brain. Moderate levels of Pgp are found in a range of other tissues also (Croop et al., 1989; Tatsuta et al., 1992). Molecules interacting with Pgp may be classified as substrates or antagonists. Cancer drugs frequently are Pgp substrates, but do not block the transport of other substrates while compounds in the antagonist or inhibitor groups, which fail to be transported, prevent the transport of other compounds.

Table 2. 1: List of Pgp subtrates

Anticancer agents	Vinca alkaloids (Vincristine, Vinblastine, Vinorelbine)
	Taxanes (Paclitaxel, Docetaxel)
	Anthracyclins (Doxorubicin, Epirubicin, Daunorubicin)
	Epipodophyllotoxins (teniposode, etoposide)
Immunosuppressives	Cyclosporine, Sirolimus, Tacrolimus
Cardiac Glycoside	Digoxin, Digitoxin, Quinidine
Glucocorticoid	Dexamathasone, Methylprednisolone
Anthelmintic	Ivermectine

HIV protease inhibitors	Saquinavir, Indinavir, Amprenavir, Ritonavir
Anticancer agents	Vinca alkaloids (Vincristine, Vinblastine, Vinorelbine) Taxanes (Paclitaxel, Docetaxel) Anthracyclins (Doxorubicin, Epirubicin, Daunorubicin) Epipodophyllotoxins (teniposode, etoposide)

Table 2. 2: List of Pgp modulators/inhibitors

1st generation drugs	Verapamil, Nifedipine, Cyclosporine, Tamoxifen, Trifluoperazine, Cremophor EL, Progesterone, Quinidine, Dipyridamole etc.
2nd generation drugs	Valspodar (PSC 833), Biricodar (VX 710), Dexverapamil, Dexniguldipine, Rapamycin
3rd generation drugs	Zosuquidar (LY335979), Laniquidar (R10193), Tariquidar (XR9576), Substituted diarylimidazole (ONT093)

Pgp has been shown to channel the ATP-mediated extrusion of MDR drugs, an activity that can be inhibited by Pgp modulators in intact cells. However, Pgp modulators do not inhibit multidrug-resistant associated protein MRP-dependent drug efflux (Cole et al., 1994). The discovery of Pgp modulators capable of reversing MDR in cells overexpressing Pgp raised hope that the administration of these modulators along with chemotherapeutics agents could overcome the MDR phenomenon. But the treatment with Pgp modulators was unsuccessful due to the fact that MDR is not solely mediated by Pgp and additional mechanisms exist which cannot be reversed by Pgp modulators (Fisher and Sikic et al 1995; Arceci, 1993; Kaye, 1995; Bates et al., 1996).

2.3 Strategies used to overcome MDR of cancer cells
In order to overcome MDR in cancer cells various strategies can be used that will inhibit or bypass (circumvent) Pgp so that the antitumor agent does not get effluxed out of the cell for an effective treatment. The different strategies that can be used to inhibit or circumvent the Pgp pump are

- Formulation of anticancer agents into colloidal drug delivery system.

- Non substrate strategy (Borowski et al., 2005)
- Design and development of cytostatics that have a fast cellular uptake which surpasses their MDR mediated efflux.
- By interfering with ATP hydrolysis (Shapiro and Ling., 1997)
- By altering integrity of cell membrane lipids (Drori et al., 1995)
- Use of modulators or inhibitors (act as substrate or bind with ATP binding sites competitively or non-competitively i.e. allosterically).
- Controlling the expression of MDR proteins
    - ✓ Antisense oligonucleotide approach
    - ✓ MDR1-specific transcription factor targeting
    - ✓ Post transcriptional gene silencing approach/RNA interference (RNAi) approach
    - ✓ Post translational modifications of the transporter proteins

2.3.1 Non-substrate strategy

The 'Non Substrate strategy' is of much importance because it enables to have a monotherapy treatment which omits the use of any other augmenting agents that are active pharmacologically. The other important advantage of this technique is that the non-substrates will not block other ABC transporters in the normal tissues. But the main problem with this technique is that finding a non-substrate is very difficult because Pgp have a broad spectrum of substrates (Borowski et al., 2005). The classes of anticancer drugs which are non-substrates of MDR proteins are the anti-metabolites. Many of the scientists have demonstrated that 5-flourouracil, 6-thioguanine, 5-flourouridine, cytarabine, methotrexate and gemcitabine are not Pgp substrates. Infact, the dideoxynucleosides are also not recognized by Pgp in the kidney transport (Berghman et al., 2001; Litman et al., 2001; Jin et al., 2005) . Some of these antimetabolites retain their cytotoxic effects towards the cells expressing the ABC proteins but also induce the overexpression of the genes encoding these proteins and hence a net effect is increase in resistance to other antitumor agents (Lu et al., 2002).

2.3.2 Development of cytostatics that have a fast cellular uptake

The design of cytostatics that are having faster cellular uptake gives the advantage of monotherapy. The most favored mechanism of drug uptake is via diffusion across the lipid membrane. The diffusion kinetic is linear with the concentration whereas the MDR transporters are of saturation type. Hence for a cytostatic to be retained within a cell, it must have a faster influx into the cell then their mediated efflux (By MDR transporter) at the transporters saturation point. In general, higher the lipophilicity of the molecule faster the drug uptake by diffusion. But other structural factors apart from lipophilicity are also found to be important in drug migration across the lipidic membrane (Borowski et al., 2005). One of such factors in the cytostatics belonging to anthraquinone and acridine group is that inclusion of one or two fused five- or six-membered heterocyclic rings makes these molecules able to overcome MDR. There have been evidences of overcoming MDR by such polycyclic compounds because of the speed of their uptake surpassing the rate of MDR transporters mediated efflux (Gobis et al., 2001; Tarasuik et al., 2002). An anticancer drug S16020-2 (Servier- a livacine derivative) has been found to be successful in by passing Pgp mediated resistance because of its rapid uptake kinetics (Pierre et al., 1998).

2.3.3 Interfering with ATP hydrolysis

The compounds that inhibit the ATP hydrolysis can also serve as good inhibitors since there are very less chances of them being transported by Pgp and these agents will be required in low concentrations. Quercetin (A flavanoid) has been shown to act by interfering with the ATPase activity. The photo affinity analogues inhibit the ATPase activity of Pgp by interacting with the lysine residue in the nucleotide binding domains. The catalytic sites in Pgp are conformationally flexible and of relatively low affinity and specificity compared to other transport ATPases which causes the inhibition of ATP hydrolysis selectively (Al-shawi et al., 1994).

2.3.4 Altering integrity of membrane lipids

Some of the commonly used surfactants act by altering the integrity of membrane lipids. The surfactant disturbs the hydrophobic environment of the Pgp which causes a change in the secondary and tertiary structure of the Pgp and thus causing loss of Pgp function.

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Hugger et al., (2002) have shown that change in the fluidity of the cell membrane by surfactants facilitate the influx of the Pgp substrates as demonstrated in Caco-2 cell line. Surfactants stand a better chance in being used to inhibit Pgp because they are already approved for use in pharmaceutical formulations. Until now reports of their trials in humans or animals have not yet been published.

2.3.5 Use of Pgp modulators

Another class of the Pgp modulators acts by an allosteric mechanism of inhibition of Pgp. One of the thioxanthene derivative (cis-(Z)-flupentixol) has been found to prevent the substrate translocation and dissociation by allosteric modulation of human Pgp. Some of the anthranillic acid derivatives have an allosteric effect on substrate recognition or ATP hydrolysis. An indolizine sulfone (SR22557) interacts with site other than substrate recognition site and affects substrate binding to Pgp (Martin et al., 1997; 1999). The most widely studied MDR modulators are the Competitive ones. These act as substrates to MDR pumps and so compete with the cytotoxic agent for binding to the substrate binding sites on the MDR transporter proteins.

2.3.6 Controlling the expression of MDR proteins

2.3.6.1 Antisense oligonucleotide approach

A more specific way of overcoming MDR is the 'antisense oligonucleotide' approach rather than using pharmacologically active MDR modulators or inhibitors. Here the downregulation of the MDR transporter proteins is affected by antisense oligonucleotides. There have been patents (Hybridon Inc. 1999 and Isis Pharmaceuticals Inc. 1999) available on this technique of suppressing Pgp expression. Here the formation of duplexes with the target mRNA occurs by complementary oligodeoxyribonucleotides (ODNs) which causes an interruption of translation and as a result of it the resistant tumor cells are transformed to non-MDR ones. The major hurdles in using this technique are that the ODNs are specific for a particular RNA target, cellular uptake is fast and are susceptible to blood serum nucleases (Walder and Walder, 1988; Crooke, 1992). Hexitol Nucleic acids (HNA) antisense oligonucleotides were delivered to MDR1 expressing cells by transfection. This anti MDR1 HNA 'gapmer' was

very potent in reducing the expression of Pgp which resulted in increased cellular accumulation of drug surrogate Rhodamine123 (Kang et al., 2004).

2.3.6.2 MDR1 specific transcription factor targeting

A repressor molecule for targeting the MDR1 specific transcription factor was found out by Bartsevich and Juliano, 2000. Efferth et al, 2001 used an antitumor agent 5-azacytidine to modify MDR1 promoter region as a result of which the transcriptional activity was inhibited and the resistance K562 cells were transformed into non-MDR type. Interesting results were found with HMN-17 (an active metabolite of antitumor agent HMN-214, a stilbazole derivative) (Tanaka et al., 2003) and also with ecteinascidin 743 an isoquinoline derivative (D'Incalci, 1998). One of the ways of targeting the mRNA is by using a ribozyme (a catalytic RNA). The anti–MDR ribozymes when introduced into the tumor cells can reverse MDR by cleavage of MDR1 mRNA (Nagata et al., 2002; Wang et al., 2003).

2.3.6.3 Post transcriptional gene silencing

In the post transcriptional gene silencing approach a small interfering RNA (siRNA) duplex (21 nucleotides) targeting the MDR1 gene is introduced into the cancer cells. The siRNA duplex causes the inhibition of the MDR1 by degrading the complementary MDR1 mRNA. This technique is found to be more potent as well as more specific. siRNAs do not have any effect on the expression of unrelated genes rather they are more specific for the expression of the gene from which their sequences are derived (Wu et al., 2003; Elbashir et al., 2001). This method has a limited use because the silencing effect on the Pgp expression is short lived i.e. not more than 24 h (Hannon, 2002). Two efficient siRNAs led to a very satisfactory Pgp extinction (only 20% Pgp expression remaining) with siRNA concentration as low as 20 nM (Stierle et al., 2004). This approach was used to reverse the MDR in human breast cancer cell line (MCF-7/AdrR). Here RNA interference was brought about by a small hairpin RNA (shRNA). Resistance against doxorubicin was found to decrease from 162 to 54-fold (transient transfection) and from 108 to 50-fold (stable transfection). Furthermore, shRNA vectors significantly enhanced the cellular daunorubicin accumulation. The combination of shRNA vectors and doxorubicin significantly induced apoptosis in MCF-7/AdrR cells (Gan et al., 2005).

2.3.6.4 Post translational modifications

Another very important technique of MDR reversal is the posttranslational modifications of the transporter proteins (here Pgp). Pgp phosphorylation by a variety of kinases helps overcome the MDR of cancer cells. When the phosphorylation occurs at the linker region, the two halves of Pgp are joined. This is the last and most important step in post translational modification and also this step is indispensable for transporter activity of Pgp. Here the protein kinases are the main targets for MDR reversal (Gupta et al., 1996; Castro et al., 1999). Sato et al., 1990 have shown the effective reversal of MDR cells by use of protein kinase inhibitors like H-87, staurosporine and their derivatives. These protein kinase inhibitors reduce the phosphorylation of the transporter protein and hence inhibit its activity (Ma et al., 1995).

2.3.6.5 Pgp specific monoclonal antibodies

Another interesting approach to overcome MDR is the use of monoclonal antibodies (MoAb). MoAb can specifically inhibit Pgp because of their high selectivity against well defined epitopes which results in abolishing the MDR phenotype of tumor cells (Mechetner and Roninson, 1992). Also the use of immunotoxins is valuable in treatment of multidrug resistant cells. Here the MoAb is coupled to anticancer agent via a linker molecule. These immunotoxins bind with the specific target antigen and exert their cytotoxic effect after internalization. They have only minimum inhibitory effect on sensitive cells. The extent of cell killing in this case has been correlated with the level of Pgp expression (Efferth and Volm, 1993).

2.3.7 Novel drug delivery systems in circumventing Pgp mediated MDR

Instead of direct inhibition of Pgp, another way of bypassing resistance is to protect the drug against the pumping action of Pgp by means of chemical modifications is by association with colloidal carriers. The rationale behind association of drugs with colloidal carriers for reversal of MDR is the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cell only when it is present in the plasma membrane and not in case when it is located in the cytoplasm or lysosomes after its endocytosis. The other advantage of using these colloidal carriers are firstly the toxic effects of the anticancer agents will be reduced due to specific and direct targeting of cancer cells. P-

glycoprotein interacts directly with non-polar substrates within the membrane environment moving drugs from the inner to the outer leaflet of the lipid bilayer (Sharom, 1997). It can be assumed that the Pgp substrates that are not able to access the transporter protein i.e. Pgp from within the plasma membrane are not recognized by Pgp efficiently. Hence the use of a drug delivery system to transport a P-glycoprotein substrate across the plasma membrane would allow to by-pass P-glycoprotein and result in intracellular drug accumulation.

2.3.7.1 Nanoparticles

Nanoparticles are solid colloidal particles ranging in size from 10 nm to 1000 nm (1 mm). They consist of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped or encapsulated and/or to which the active principle is adsorbed or attached (Kreuter 1983). The most promising application of nanoparticles is their possible use as carriers for antitumor agents (Kreuter, 1991). Drugs loaded in the nanoparticles are effective in a number of chemotherapy refractory cancers in animal as well as clinical models (Kubaik et al., 1989). It was also assumed that the nanoparticles protect the drug from the action of Pgp as they enter the cell by endocytosis.

In a recent study by Aouali and co workers doxorubicin nanospheres of polyisohexylcyanoacrylate (NS-DOX) were studied on human breast adenocarcinoma MCF7/WT cells and DOX- resistant cells (MCF7/DOX) overexpressing Pgp (Schnieder et al., 1994). It was observed that NS-DOX reversed the resistance in MCF7/DOX without restoring the nuclear accumulation of doxorubicin. A significant increase in the tumor uptake of etoposide was observed by administering etoposide loaded tripalmitin nanoparticles in comparison with the free drug. It helps in controlling the biodistribution of etoposide which ultimately results in reducing the systemic toxicity of etoposide (Reddy et al., 2005). A significantly higher tumor concentration of doxorubicin loaded poly(butyl cyanoacrylates) was observed compared to the free doxorubicin (due to reduced Pgp efflux) which lead to effective doxorubicin mediated apoptosis induction in cells causing enhanced reduction in cell density of tumor tissues (Reddy et al., 2004).
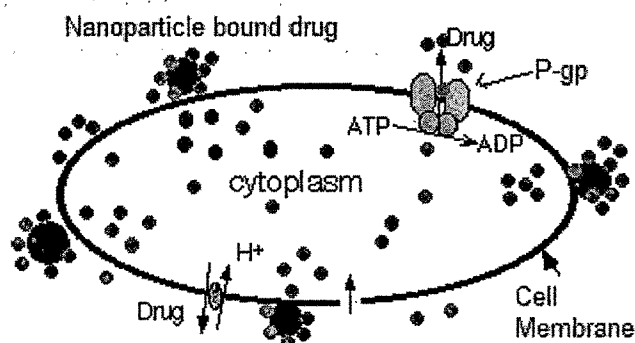
Figure 2. 3: Nanoparticle bound drug is retained in the cytoplasm

It has been demonstrated that entrapment of paclitaxel in nanoparticles of cetyl alcohol/ polysorbate significantly increases the drug brain uptake and its toxicity toward Pgp expressing tumor cells. It was hypothesized that paclitaxel nanoparticles could mask paclitaxel characteristics and thus limit its binding to Pgp, which consequently would lead to higher brain and tumor cell uptake of the otherwise effluxed drug (Koziara et al., 2004).

It was observed that doxorubicin incorporated in the non-biodegradable polymethacrylate nanospheres inhibited MDR of tumor cells but it differed from doxorubicin incorporated in PACA nanoparticles. It is assumed that doxorubicin adsorbed on to the polymethacrylate nanospheres was cell internalized by an endocytic process in U937 cells (human monocyte-like cancer cell line expressing a Pgp) and in cultured rat hepatocytes. After the cell internalization a sustained release of doxorubicin from nanospheres occurred. Because of this sustained release a slower efflux of doxorubicin and a higher intracellular accumulation and increased cytotoxicity was observed compared to the free drug. The use of this system is limited owing to the non-biodegradable nature of polymethylacrylate (Doat et al., 1988).

Other strategies that are used to bypass Pgp mediated MDR is the use of Stealth™ polycyanoacrylate nanoparticles or co-administration of doxorubicin with chemo-sensitizing agents, generally acting as Pgp inhibitors. There was an improvement in the

cytotoxicity of the anticancer agent on resistant DC3F AD/AZA subline (Chinese hamster lung cell line) when MDR reversal agents like verapamil and amiodarone were added to the cell culture medium, in combination with doxorubicin incorporated into PIBCA or PIHCA nanoparticles. But one major drawback of this strategy was that the doses of the modulators that were used were not compatible for human administration (Couvreur et al., 1990). Because of this the coencapsulation of MDR reversing agent Cyclosporine A and the anticancer agent doxorubicin was experimented. By the use of this approach the side-effects of both the drugs could be reduced with an enhancement in their efficacy. In resistant cell culture experiments it could be demonstrated that the association of both Cyclosporine A and doxorubicin within a single nanoparticulate formulation exhibited the most effective growth rate inhibition in comparison with other combinations of both drugs while using a lower amount of polymer compared to separated nanoparticles formulations of cyclosporine A and doxorubicin. The high efficacy of the combined nanoparticles formulation may be attributed from the synergistic effect due to rapid release of both the drugs at the surface of the cancerous cell which allows a better internalization of doxorubicin, while inhibiting its efflux by blockage of Pgp by cyclosporine A (Soma et al., 2000). The release of the drug from the PACA nanoparticles occurs as a result of the polymer biodegradation which renders the release profile of an entrapped compound independent of its physiocochemical characteristics, which make them the carrier of choice for treatment MDR tumors (Muller et al., 1990).

Zhang et al., 2008 have demonstrated that lipid matrix-based Nanostructured lipid carriers (NLC) can increase the drug transport into cancer cells, and overcome the multi-drug resistance. The reversal power in multi-drug resistant cells was improved when the NLC was modified with folic acid, which revealed a potential application for reversing multi-drug resistance of human cancer cells. Garcion et al., 2006 have demonstrated the effectiveness of NLC in overcoming the overall MDR mechanisms.

Pluronics® have been widely used in resistant cancers. One of the major reasons for enhanced cytotoxicity with Pluronics® in drug resistant cancer appears to be related to the effects of the copolymer on the Pgp drug efflux transport systems. The use of

Pluronic® block copolymers to treat drug-resistant cancers is a rapidly developing area of drug delivery for cancer chemotherapy. Studies by Alakhov et al. demonstrated that Pluronic® block copolymers sensitize resistant cancer cell lines, reulting in an increase in the cytotoxic activity of the drug by 2 to 3 orders of magnitude (Alakhov et al., 1996; Venne et al., 1996; Batrakova et al., 1999). By addition of P85 or L61, the cytotoxic effects of doxorubicin in the resistant lines significantly surpassed those observed in the sensitive lines.

2.4 Drug targeting

Nanoparticles can be used to decrease the toxicity of the drug to non-target organs by targeting the drug to the tumor tissue either by passive targeting or by active targeting. A site-specific delivery would not only increase the amount of the drug reaching the site but also decrease the amount being distributed to other parts of the body, thus reducing unwanted site effect. Site-specific or targeted delivery, therefore, would also enable a reduction in the dose to be administrated. By decreasing side effects, it would also increase the therapeutic index of the drug.

Passive targeting is defined as a method whereby the physical and chemical properties of carrier systems increase the target/nontarget ratio of the quantity of drug delivered by adjusting these properties to the physiological and the histological characteristics of the target and non-target tissues, organs, and cells. Influential characteristics of passive targeting are chemical factors such as hydrophilicity/hydrophobicity and positive/negative charge and physical factors such as size and mass. *Active targeting* refers to efforts to increase the delivery of drugs to a target through the use of specific interactions at target sites where a drug's pharmacological activities are required.

Colloidal systems like nanoparticles and liposomes possess the tendency to reach some type of tumour after intravenous injection. This behaviour makes the colloidal systems a suitable carrier for cytostatic drugs. The reason of this behaviour is not fully explained so far, but it is indisputable that the increase of the tissue permeability and the adherence of particles to the tissues play a relevant role (Luo and Prestwich 2002; McDonald and Baluk 2002). Furthermore, the growth of the tumour is associated to inflammatory

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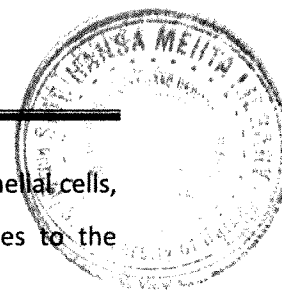
reactions, and this phenomenon leads to the enhanced endocytosis by endothelial cells, to the fenestration of endothelium, and to the migration of T-lymphocytes to the enflamed tissues (Mahaley 1968; Giometto et al. 1996; Tran et al. 1998).

2.5 Enhanced permeation and retention effect

Differences in the structure and behaviour of normal and tumour tissue could be used for designing drug delivery systems facilitating tumour-specific delivery of the drug. Generally, three locations in the tumour tissue are used as targets for delivery of anti-cancer drugs in drug delivery research: tumour vasculature, extracellular space in the tumour tissue, and tumour cells. In principle, accumulation of polymer-based drugs in many tumours can be achieved by a nonspecific (passive) or by a specific targeting (active) process. Tumour vasculature continuously undergoes angiogenesis to provide blood supply that feeds the growing tumour (Hanahan and Folkman 1996). High-molecular-weight molecules and nano-sized particles accumulate in solid tumours at much higher concentrations than in normal tissues or organs due to the enhanced permeability and retention (EPR) effect (Maeda 2001; Seymour et al. 1995; Maeda et al. 2000, 2003). In this case, a leaky vasculature and limited lymphatic drainage, typical of tumour and missing in normal tissue, result in the accumulation of macromolecules, e.g. macromolecular drug carrier systems in the interstitial space of a large variety of tumours (Maeda 2001; Maeda et al 1992, 2001) (Figure 2.4). These systems can release cytotoxic drugs into the extracellular fluid of the tumour tissue (Christie and Grainger 2003; Takakura and Hashida 1995), or they can release a drug after entering the tumor cells via endocytosis (Alberts et al., 2002).
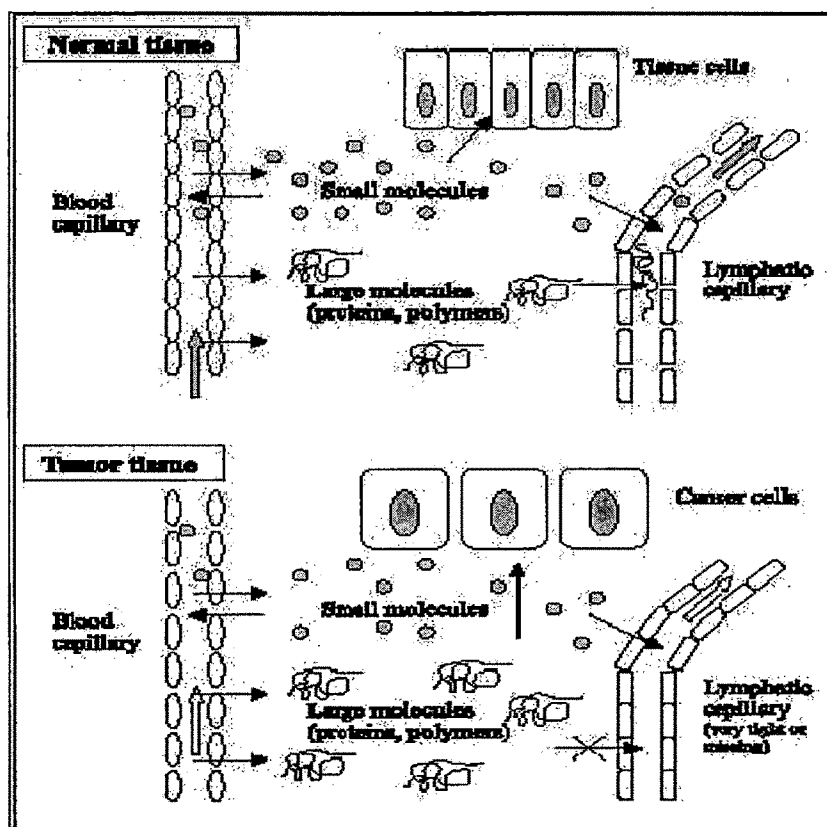
Figure 2. 4: Schematic illustration of the EPR effect principle. Angiogenesis and enhanced vascular permeability of tumour capillaries and impaired or missing lymphatic clearance of macromolecules result in accumulation of macromolecules (polymers) in tumour tissue.

2.6 Drug Profile

Paclitaxel (PTX) is a diterpenoid pseudoalkaloid derived from the needles and bark of the Pacific yew tree (*Taxus brevifolia*; family Taxaceae) having antineoplastic activity against a wide range of cancers particularly against primary epithelial ovarian carcinoma, breast cancer, colon, head and neck cancers and non-small cell lung cancer.   It is a potent inhibitor of cell replication, blocking cells in the late G2-mitotic phase of the cell cycle by stabilizing the microtubule skeleton.

2.6.1 Physicochemical properties

   a. **Appearance:** White to off-white crystalline powder

   b. **Category:** Antineoplastic

   c. **Generic name:** Paclitaxel

d. **IUPAC name:** 5β,20-Epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)- N-benzoyl-phenylisoserine.

e. **Marketed preparations available: Taxol®** by Bristol Myers Squibb, US; **Abraxane®** American Bioscience Inc., USA approved on Jan 2005; albumin bound paclitaxel; **Nanoxel®** by Dabur Pharma, India

f. **Empirical formula:** C47H51NO14

g. **Molecular weight:** 853.91.

h. **Melting point:** 216°C to 217°C.

i. **Solubility:** Insoluble in water, freely soluble in alcohol

j. **Structure:**
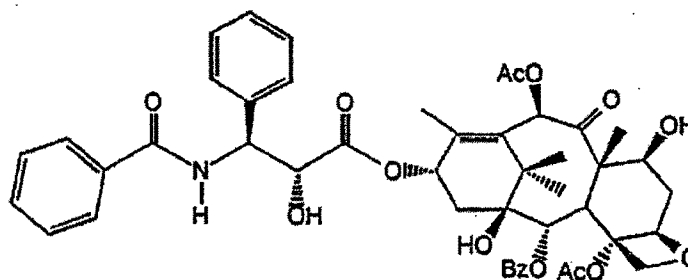
2.6.2. Pharmacology (mechanism of action)

PTX promotes the polymerization of tubulin. The microtubules formed in presence of PTX are extraordinarily stable and dysfunctional, thereby causing the death of the cell by disrupting the normal tubule dynamics required for cell division and vital interphase process. In addition, paclitaxel induces abnormal arrays or bundles of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis (Schiff et al., 1979; Hamel et al., 1981; Parness and Horwitz, 1981; Rowinsky et al., 1990).

2.6.3 Pharmacokinetics

Following intravenous administration, paclitaxel exhibits a biphasic decline in plasma concentrations with a mean terminal half life of between 3 and 50 hrs. The pharmacokinetics of paclitaxel are non-linear. There is a disproportionately large increase in Cmax and AUC with increasing dose, accompanied by an apparent dose-

related decrease in total body clearance. On average, 89% of drug is bound to plasma proteins. After i.v. administration, mean values of cumulative urinary recovery of unchanged drug ranged from 1.3 to 12.6% of the dose, indicating extensive non-renal clearance. Hepatic metabolism and biliary clearance may be the principal mechanism for disposition of paclitaxel. It is metabolized primarily by cytochrome P450 isoenzyme CYP2C8 although CYP3A4 may play a minor role. Hydroxylated metabolites have been demonstrated to be the principal metabolites that are excreted in the faeces via bile. The formation of 6 alpha-hydroxypaclitaxel, 3'-p-hydroxypaclitaxel and 6 alpha,3'-p dihydroxypaclitaxel is catalysed by CYP2C8, 3A4 and both 2C8 and 3A4 respectively.

2.6.4 Toxicology

<u>Side-Effects</u>

Bone marrow suppression and peripheral neuropathy are the principal dose-related adverse effects associated with TAXOL. Myelosuppression is less frequent and less severe.

<u>Hematologic:</u>

Neutropenia is very common and is generally rapidly reversible. Thrombocytopenia occurs in a few patients. Anaemia has been observed in the majority of the patients. Incidence and severity of anaemia is related to baseline haemoglobin status. TAXOL therapy should not be administered to patients with baseline neutrophil counts of < 1,500 cells/mm^3. Patients should not be retreated with subsequent cycles of TAXOL until neutrophils recover to a level >1,500 cells/mm^3 and platelets recover to a level >100,000 cells/mm^3.

<u>Hypersensitivity Reactions:</u>

Hypersensitivity reactions may occur requiring therapeutic intervention and/or early discontinuation of TAXOL infusion despite premedication. Dyspnoea, flushing, chest pains and tachycardias are the most frequent manifestations. Minor manifestations are flushing, rash and hypotension.

<u>Cardiovascular:</u>

Hypotension and bradycardia have been observed during administration of TAXOL. Cases of myocardial infarction have been reported. Congestive heart failure has been reported typically in patients who have received other chemotherapy, notably

anthracyclines. Hypertension, venous thrombosis, ventricular tachycardia, and atrioventricular conduction block. ECG alterations are experienced by some patients.

Neurological:

Peripheral neuropathy occurs and is dose dependent. Besides peripheral neuropathy, other rare neurologic manifestations are grand mal seizure, syncope, ataxia and neuroencephalopathy. Reports of motor neuropathy with resultant minor distal weakness and autonomic neuropathy resulting in paralytic ileus and orthostatic hypotension have appeared. Optic nerve and/or visual disturbances (scintillating scotomata) have also been reported, particularly in patients who have received higher doses than recommended. These effects generally have been reversible. Ototoxicity has been reported.

Arthralgia/Myalgia:

Arthralgia/myalgia usually consisting of pain in the large joints of the arms and legs occurs but is usually mild.

Hepatic:

Hepatic necrosis and hepatic encephalopathy leading to death have been reported.

Other Clinical Events:

Alopecia has been observed in almost all of the patients. Transient and mild nail and skin changes have been observed. Gastrointestinal side effects such as nausea/vomiting, diarrhoea and mucositis have been reported. Neutropenic enterocolitis has been reported. Extravasation during intravenous administration may lead to oedema, pain, erythema and induration and ulceration Bowel obstructions/perforations and ischemic colitis have been reported in patients treated with paclitaxel.

2.6.5 Indications

1. The palliative treatment of stage 3 or 4 advanced local carcinoma of the ovary after surgical resection, in combination with cisplatin.

2. The palliative management of metastatic carcinoma of the ovary after failure of first line or subsequent chemotherapy.

3. The treatment of metastatic carcinoma of the breast after failure of combination chemotherapy or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contra-indicated.

4. Palliative treatment of advanced non-small cell lung cancer in patients who are not candidates for potentially curative surgery and/or radiation therapy.

2.6.6 Dosage and administration

Indication 1:

Primary treatment of ovarian carcinoma: a combination regimen consisting of TAXOL 135 mg/m^2 administered over 24 hours, followed by cisplatin 75 mg/m^2, every 3 weeks. TAXOL should be administered before cisplatin.

Indication 2 and 3:

Secondary treatment of ovarian carcinoma: TAXOL at a dose of 175 mg/m^2 administered intravenously over 3 hours every 3 weeks has been shown to be effective in patients with metastatic carcinoma of the ovary or breast after the failure of first line or subsequent chemotherapy.

Indication 4:

Palliative treatment of advanced non-small cell lung carcinoma: the recommended dose of Taxol is 175 mg/m^2 administered over a period of 3 hours; followed by a platinum compound, with a 3 week interval between courses. All patients must be premedicated with corticosteroids, antihistamines, and H2 antagonists prior to TAXOL administration, TAXOL should be administered through an in-line filter with a microporous membrane not greater that 0.22microns.

2.6.7 Paediatric use, pregnancy and lactation

The safety and effectiveness of TAXOL in children have not been established. TAXOL has been shown to be embryotoxic, foetotoxic and to decrease fertility in animal studies. There is no information on the use of TAXOL in pregnant women. TAXOL may cause foetal harm when administered to pregnant women. TAXOL should not be used during pregnancy. Women of childbearing potential should be advised to avoid becoming pregnant during therapy with TAXOL, and to inform the treating physician immediately should this occur. Breast feeding should be discontinued for the duration of TAXOL therapy.

2.6.8 Contraindications

PTX is contraindicated in patients who have a history of severe hypersensitivity reactions to TAXOL or other drugs formulated with polyoxyethylated castor oil. TAXOL should not be used in patients with baseline neutrophils <1 500/mm^3.

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