1. INTRODUCTION

Oncologic therapy could have different aims. Whenever it is possible, the main purpose of every oncologic therapy is to be curative - in the sense of achievement of a permanent tumor-free status. There are three main approaches in cancer therapy: surgery, chemotherapy and radiotherapy. Nowadays, the multimodal approach is used in the management of malignant diseases. Chemotherapy and radiotherapy are carried out as adjuvant and additive after surgery, but also as primary treatment. Although most tumors respond to initial treatment, recurrences are common and they no longer could be treated with chemotherapy. The underlying mechanism of this phenomenon is known as drug resistance and is part of the evolutionary process, in which the organisms had to develop systems that allow them to withstand the continuous exposure to noxious chemicals in the environment. Understanding the molecular mechanisms of drug resistance is fundamental to medical science and would allow new treatment strategies to be developed where this particular problem does not arise. The achievement of even a modest therapeutic advantage can sometimes have a significant influence on survival in cancer patients. The circumvention of drug resistance would allow development of strategies which are one hundred percent efficient on initial use, and do not permit survival of subpopulations of target cells.

1.1. Cell resistance to chemotherapeutic drugs

In the recent years, major advances have been made in the chemotherapy treatment of hematology malignancies and germ cell tumors. In solid tumors, response rates as high as 90 % could be achieved in certain histological subtypes such as small cell lung cancer; however, cures are rare and the disease invariably recurs. The main obstacle is that tumors may possess an intrinsic, prior to treatment resistance or drug resistance acquired during treatment of tumors. Such a tumor intrinsic property is common of non-small cell

lung cancer, while small cell lung cancer typically acquires resistance only after treatment of the patient with single or multiple agent chemotherapy. The tumors that acquire resistance to therapy are by definition initially responsive to conventional chemotherapy. However, even in these responsive tumor types, disease recurs and curative therapy remains elusive. Upon recurrence, the disease progression is usually significantly altered in that responses to therapy are either absent or shorter in duration. Ultimately, these tumors are completely refractory to cytotoxic drugs and the patients can no longer be treated with conventional chemotherapy. Cancer cell resistance can occur at many levels, including increased drug efflux (as exemplified by ATP binding cassette transporters - ABC) or decreased influx. Once inside the cell, the activation of the drug can be impaired or can be enzymatically inactivated. Cellular targets can be altered or the drug can be redistributed inside the cell (e.g. vaults). Once a cellular target is damaged, the apoptosis can be attenuated (Fig.1.1.1) (Baird RD et al., 2003; Kruh GD et al., 2003; Ling V et al., 1997; Longley DB et al., 2005). However, drug resistance in cancer could be also dependent on tissue heterogeneity, drug delivery, tissue oxygenation and cell growth rate. It is possible to select cells that simultaneously acquire resistance to a range of structurally and functionally unrelated cytotoxic drugs following exposure to only one cytotoxic agent – this is known as multidrug resistance. Two types of multidrug resistance phenotype have been characterized following such a selection. The first is alteration in a single key target important for the action of various compounds and is typical of the resistance to anthracyclines, epipodophyllotoxins and aminoacridines. The second type involves classes of structurally unrelated drugs with different intracellular targets and mechanisms of action, such as vinca alkaloids and taxol derivates, but also anthracyclines. This profile of cross resistance has been commonly used to define a so-called classical or typical multidrug resistance phenotype.

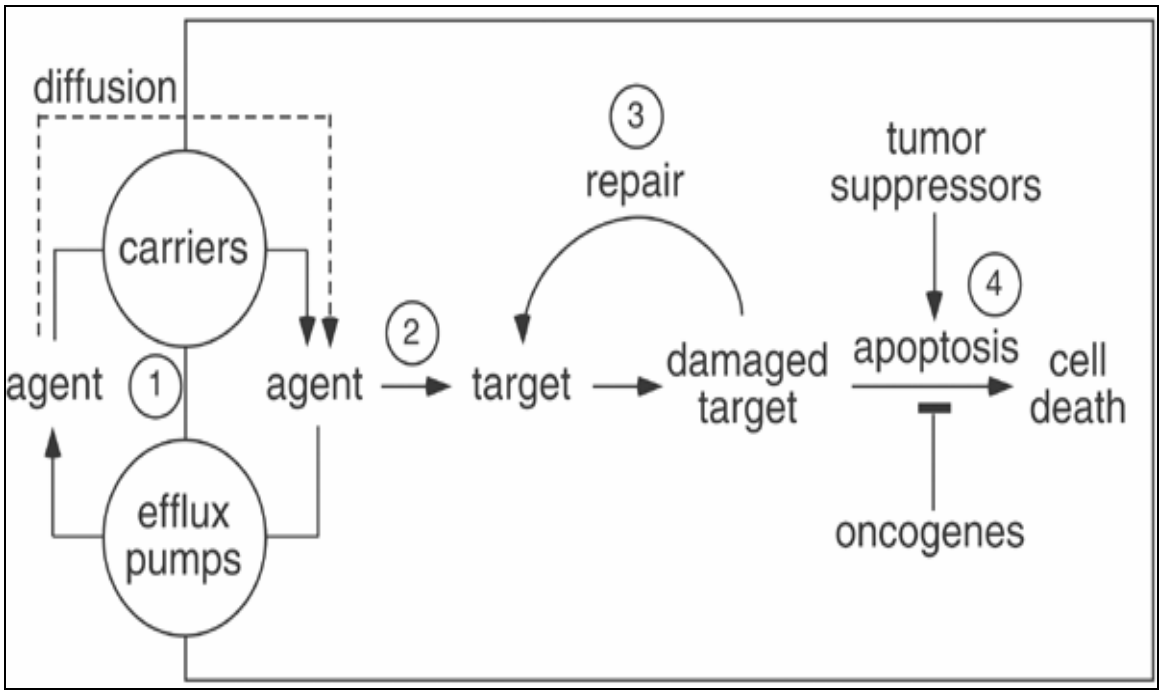

Fig. 1.1.1. A classification of the cellular drug resistance-associated mechanisms. The drug is extruded out of the cytoplasm (1). The drug is extruded out of the nucleus (2); the drug could not reach the target, i.e., DNA. Repair of a cell target (3) and suppression of the apoptosis (4).

1.2. Bendamustine, cisplatin and doxorubicin as an option in the treatment of cancer

Cisplatin is one of the most widely used alkylating agents. Those drugs are thought to enter the cell, react with the DNA and trigger apoptosis and cell death. Knowledge of the mechanisms of action of cisplatin has improved our understanding of the resistance to it. The action of cisplatin in the cell could be attenuated through many pathways. However, the drug efflux out of the nucleus and the cell are of major importance (Kartalou et al., 2001). Cisplatin is not a common substrate of the P-glycoprotein, but it is pumped out of the cell in ATP-dependent fashion. However, recently has been proved that the multidrug resistance-associated proteins (MRPs) have a high affinity to cisplatin (Wernij et al., 2004). The lung resistance protein (LRP) is also known to extrude cisplatin out of the nucleus (Berger et al, 2000+2005, Mossink et al, 2003 1+2). However, little is known

about some novel alkylating agents as bendamustine and about the mechanisms leading to the occurrence of resistance to them.

The group of topoisomerase inhibitors and its subgroup of the anthracyclines are exemplified by doxorubicin. There is a diversity of mechanisms determining the resistance to anthracyclines in tumor cells. The classical multidrug resistance phenotype due to the expression of P-glycoprotein is a well-characterized phenomenon in the resistance to anthracyclines (Nielsen et al., 1996). However, a non-classical multidrug resistance phenomenon to anthracyclines due to the expression of MRPs and LRP is well known, too (Kartalou et al., 2001; Nielsen et al., 2001).

1.3. The role of radiotherapy in the appearance of chemoresistance

In the modern management of malignant diseases, the combined approach of chemotherapy and radiotherapy is commonly used. Thus, it is very important to be designed clinical schedules for maximal therapeutic effect. The main objective of experimental studies in this area is to define the synergetic drug-radiation interactions, which would lead to clinical benefits. There are many factors that influence interactions between radiotherapy and chemotherapy, including tumor type, drug characteristics and the schedule of administration, but also radiation dose, its fractionation, as well as chemotherapy and radiation therapy sequence.

Simultaneous resistance of tumors to multiple cytotoxic drugs is a major limitation to successful cancer treatment. It is widely known that radiation may interact with single drugs in several ways resulting in sub-additive, additive or supra-additive responses. These definitions are gradually being applied to interactions between acute X-irradiation and drug combinations, fractionated X-irradiation together with single or combined drugs. It has also been observed that patients with previous radiotherapy had a lower rate of response to chemotherapy (Shaw et al., 1978). This led to a number of studies showing clearly that the exposure of tumor lines of rodent or human origin to fractionated irradiation in vitro resulted in significant alterations in their drug responses (Dempke et al., 1991+1992; Hill et al., 1988+1990; McClean et al., 1993 1+2). Despite the clinical

importance of this association, there are few cellular models by means of which this relationship is studied. Most experimental investigations of drug-irradiation interactions involve single exposures to radiation, whereas in clinical practice, radiotherapy is generally delivered according to a fractionated protocol. This obstacle could have limited the researchers to extrapolate the experimental data onto clinical practice. However, Hill et al. described the identification of P-glycoprotein overexpression following X-irradiation, associated with drug resistance (Hill et al., 1990) and that occurred despite of a lack of gene amplification or of significant alterations in mdr1 mRNA levels (Dean et al., 2001). It seems that decreased drug accumulation is the most frequently reported reason for drug resistance after irradiation (Andersson et al., 2002). It occurs when a lipophilic drug selects cells for drug resistance, increasing the expression of the mdr1 gene product, the P-glycoprotein, which participates in drug efflux, and causes the multidrug resistance phenotype (Kane et al., 1996). P-glycoprotein overexpression was also observed in vivo, in experiments with human lung carcinoma xenografts (Mattern et al., 1991), and was followed by studies showing that irradiation could increase P-glycoprotein levels in Chinese hamster ovary cancer cells (Mattern et al., 1993), breast cancer (Akahi-Tanaka et al., 1995; Wazer et al., 1993), pancreatic cancer (Lee et al., 1999), ovarian cancer (Hill et al., 1991+2000), brain endothelial cells (Andersson et al., 2002) and nasopharyngeal cancer (Bu et al., 2004). That phenomenon coincided with detectable levels of resistance to cisplatin and doxorubicin, as well as ethoposide, vinorelbine, vincristine (Caney et al., 1999+2000; Dempke et al., 1991; Hill et al., 1990+2000; Locke et al., 2003; Nielsen et al., 2001). The multidrug resistant phenotype was detected for a period exceeding 6 months after completion of irradiation (Henness et al., 2002). Recently, it was described that MRP1, another transporter (Low et al., 1996), is upregulated following gamma-irradiation and this phenomenon is accompanied by a resistance to vepeside and vincristine (Harvie et al., 1997; Nielsen et al., 2001). Another study has established that only 5 % of the patients with oral squamous cell cancer had a significant P-glycoprotein expression before irradiation versus 72 % after irradiation (Ng et al., 1998). A series of studies connected the occurring resistance after gamma-irradiation with upregulation of other resistance related proteins such as glutathione-S-peroxidase-pi, topoisomerase-2-alfa, thymidilate synthetase, heat shock protein 70, but

their role is still contradictory (Eichholtz et al., 1993; Henness et al., 2004; Stammler et al., 1996). However, in order to be overcome the appearance of multidrug resistance after irradiation, a new approach is under study - simultaneous use of radiotherapy and chemotherapy. Nevertheless, there is no investigation if the multidrug resistance genes are upregulated during such a treatment especially at very low doses of the radiotherapy schedules.

In conclusion, it is obvious that some ATP-binding cassette (ABC) drug transporters are overexpressed after gamma-irradiation and it becomes apparent that the interaction between drugs and irradiation is one of immense complexity, but crucial for a successful therapeutic schedule. However, there are no investigations if such an upregulation occurs in colorectal cancer, where the adjuvant radio-chemotherapeutic approach is common.

1.4. Multidrug resistance proteins and the multidrug resistance phenotype

1.4.1. P-glycoprotein

ATP-binding cassette transporters are a superfamily of 48 integral proteins, grouped in seven families, ranging from A to G (Dean et al., 2001; Szakacz et al., 2004). They are overexpressed in a number of human cancers, including leukemia and solid tumors. In ovarian cancer, up to two-thirds of tumor specimens had been found to overexpress P-glycoprotein and this usually correlated with a poor overall survival (Gottesman et al., 2002). ABCB1 (MDR1/P-gp), a representative of an 11 member subfamily of the ABCB proteins (MDR-TAP), was first identified to be attributed to the multidrug resistance phenotype (Juliano RL et al., 1976).

The 170-kDa P-glycoprotein consists of 1280 amino acid residues and is a product of the 120 kb mdr1 gene (Ueda et al., 1986) located on the 7q21 chromosome. It has two homologue halves, each of a hydrophilic cytoplasm part, with ATP-binding capacity and a hydrophilic one. The latter, with twelve transmembrane segments, forms twelve angled, 5 nm big membrane pores conforming a transmembrane channel, through which

substrates are carried out of the cell against the gradient utilizing ATP (adenosine triphosphate) as an energy source. Its activity is regulated by cAMP (cyclic adenosine monophosphate), cGMP (guanosine monophosphate) dependent protein kinases, which on their behalf are calcium dependent. P-glycoprotein plays an important role in the blood-tissue barriers (Ling V, 1997) such as in the capillaries of the brain, testis, ovaries, and placenta. In the epithelial cells of the gastrointestinal tract, kidneys, bronchia, mammal glands and liver P-glycoprotein extrudes cell metabolic products as well as xenobiotics and their metabolites out of the body and in that way also promotes drug resistance. The overexpression of P-glycoprotein in mammals is usually due to a gene amplification. Although expression of mdr1 is often examined, its regulatory mechanisms are poorly understood (Sukhai et al., 2000). Evidence indicates that expression could be controlled pre- or post-transcriptional by a myriad of environmental mechanisms (Fig. 1.4.1.1).

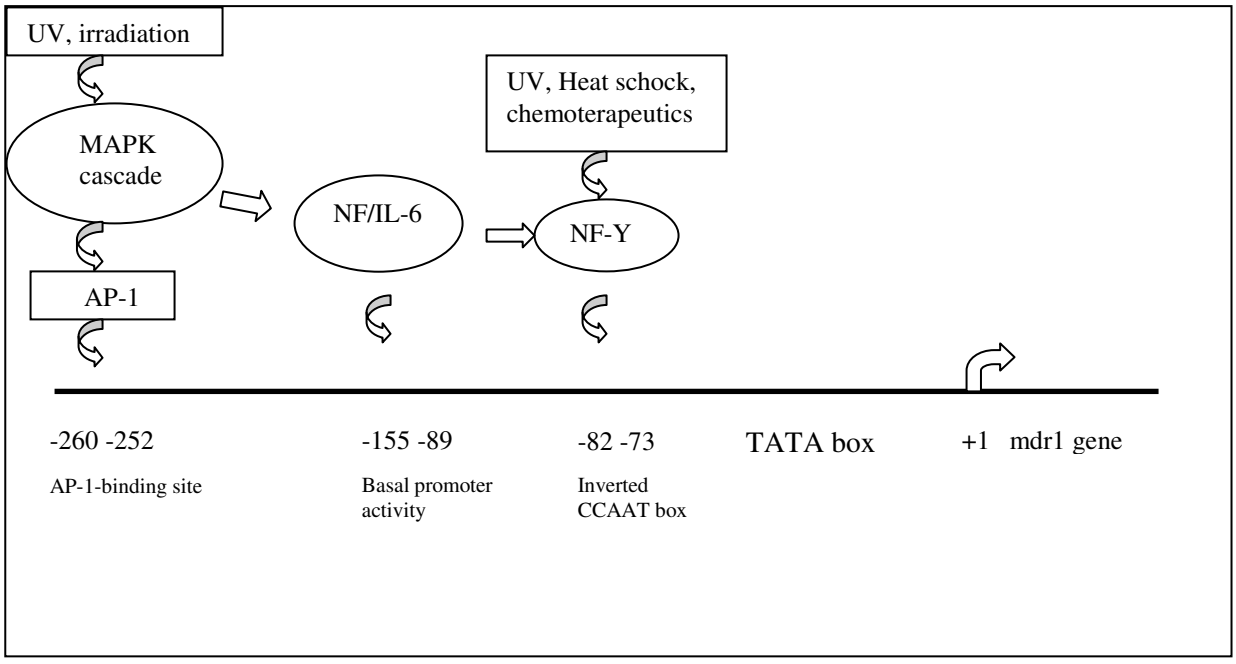

Fig. 1.4.1.1. A possible way of mdr1 mRNA upregulation by environmental stimuli, including irradiation. NF- Necrosis Factor, UV – ultraviolet light, MAPK - Mitogen-Activated Protein Kinase, AP-1 – Activator Protein-1, IL-6 – interleucin 6.

Modulation in protein stability, plasma membrane incorporation, mRNA stability and processing, gene transcription and gene amplification has been reported (Chin et al.2003; Jin et al., 1998). Fractionated irradiation has also been reported to increase P-glycoprotein expression in Chinese hamster ovary (CHO) cancer cells due to an increased protein stability and half-life with corresponding decreases in turnover rates, so the half-lives of P-glycoprotein was increased by up to 40 hours in the irradiated cells compared to 17 hours in those treated with chemotherapy alone. Moreover, that occurred without concomitant increases of mRNA level (McClean et al., 1993). Recent evidence suggests that chromatin superstructure plays a role in transcriptional regulation during irradiation. Substrates of P-glycoprotein are compounds of different structures such as vinca alkaloids, anthracyclines as doxorubicin (Cheng et al., 2000; Nielsen et al., 1996), as well as epipodophylotoxins, mitomycin C, actinomycin D, taxols, mythramycin (Germann et al., 1996; Szakacs et al., 2004) and others (Table 1.4.1.1.).

Possible ways to overcome P-glycoprotein mediated resistance are calcium antagonists from the verapamil group, cyclosporine A, calmoduline inhibitors, isoprenoids, tamoxifen, progesterone, ketotifen, epigallocatechin, valspodar and phenothiasines (Chen et al., 2005; Krishna et al., 2000; Kruh et al., 2003; Qadir et al., 2005; Quian et al, 2005).

Anticancer drugs	**Other drugs**	**Reversing agents**	**Peptides**
Actinomycin D	Colchicine	Amiodarone	ActinomycinD
Daunorubicin	Emetine	Cyclosporine A	Grandamycin
Doxorubicin	Ethidium bromide	Epigallocatechin	Valinomycin
Etoposide	Mithramycin	Ketotifen	
Isoprenoides	Puromycin	Niffedipin	
Mitomycin C		Phenothiazines	
Mitoxantrone		PK-104	
Paclitaxel		Progesteron	
Taxols		Reserpine	
Teniposide		Tamoxifen	
Topotecan		Valspodar	
Vinblastine		Verapamil	

Table 1.4.1.1. Compounds interacting with P-glycoprotein, including possible reversing agents.

1.4.2. Multidrug resistance protein 1 (MRP1)

MRP1 is one of a nine-member ABC transport proteins subfamily (Table 1.4.2.1). It is a 190-kDa protein, a product of 1531 kb long gene (mrp1 gene), found in humans in the 16th chromosome. The amino-acid sequence of MRP1 resembles P-glycoprotein to a modest extend (15%), and is distinct as well. MRP1 is composed of a core segment similar to P-glycoprotein's, but has an additional third membrane spanning domain in which five transmembrane helices reside. Each of the two hydrophobic transmembrane domains of the core is composed of six helices and a linker segment (Fig. 1.4.2.1). The hydrophilic part has ATP-binding capacity and is localized on the inner cellular membrane (Loe et al., 1996). In spite of the similarity in the resistance profiles of P-glycoprotein and MRP1, the substrate selectivity of the two pumps differ markedly in that

P-glycoprotein substrates are neutral or mildly positive lipophilic compounds whereas MRP1 is able to transport lipophilic anions such as glutathione, glucoronate and sulphate conjugates. However, it is still disputable if MRP1, together with MRP2 and LRP, actually takes part in the extrusion of the platina out of the cell. It was proved that deficiency of the pump is associated with a modest sensitization to taxanes and mitoxantrone, which are distinguished as notable substrates of the P-glycoprotein. In contrast to P-glycoprotein, which extrudes xenobiotics into bile, intestine and blood for terminal elimination out of the body, MRP1 is a basolateral transporter, i.e., it moves the substrate away from the luminal surfaces into tissues beneath the basal membrane.

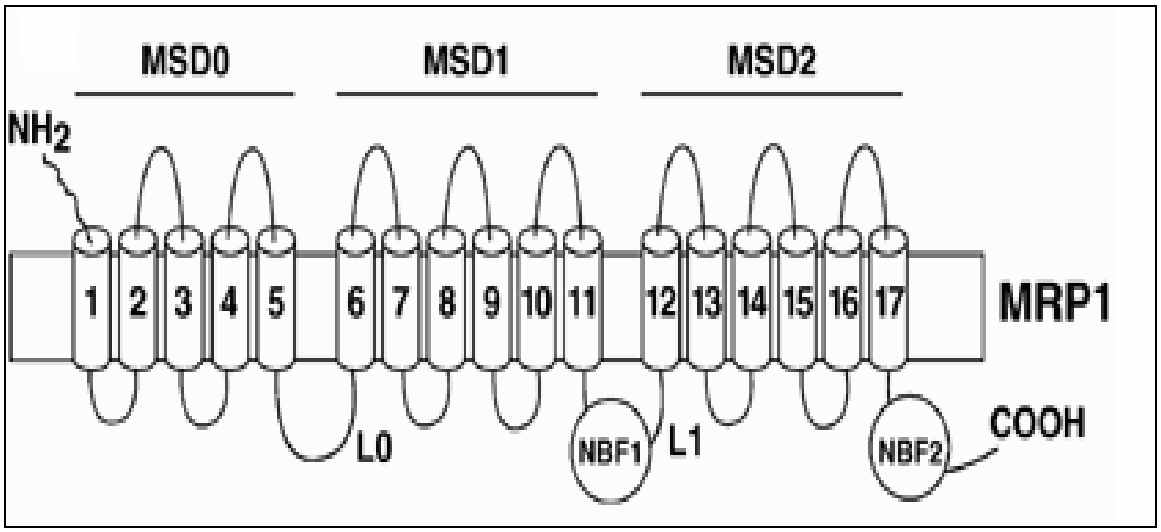

Fig. 1.4.2.1. Schematic outlook of MRP. MSD0, MSD1 and MSD2 are the membrane domains. L0 and L1 are the linker regions.

Notable physiological substrates are the metabolites of the arachidonic acid, esp. LTC4 (leucotriene C4), involved in the late anaphylactic reaction (Kruh et al., 2003). A high expression can be found in the suprarenal glands, testis, liver, lungs and colon. Little is known about the regulation pathways of MRP1; however, it seems that gamma-irradiation can cause an increase in mrp1 mRNA as well as MRP1 (Harvie et al., 1997).

Protein	Conjugates	GSH	Resistance profile	Physiological substrates	Physiological functions
MRP1	+	+	Anth, Vinc, Etop, Platina (?)	LTC4	GS-X pump, immune response
MRP2	+	+	Anth, Vinc, Etop, Camp, MTX, Platina (?)	Bilirubin glucoronate	Hepatobiliary extrusion of amphipatic anions
MRP3	+	+	Etop, MTX	Glycocholic acid	?
MRP4	+	+	6-MP	Cyclic nucleotides	?
MRP5	+	+	6-MP	Cyclic nucleotides	?
MRP6	+	+	Anth, Etop	?	Elastic tissue
MRP7	+	+	?	?	?
MRP8	?	?	5-FU	PMEA	?
MRP9	?	?	?	?	?

Table 1.4.2.1. Summary of MRP family members - resistance profiles and notable substrates.

MRP1 resistance had been reported to be overcome by calcium antagonists from the dihydropiridine group, but also amiodarone and genistein. In MRP1- overexpressing cell lines, a glutathione synthesis inhibitor, buthionine sulphoximine (Davey et al., 1996), also restored drug sensitivity. This suggests that MRP1, in conjunction with glutathione, causes multidrug resistance phenotype by drug efflux.

1.4.3. Lung resistance protein (LRP)

In 1993, an Mr 110 000 protein was found to be overexpressed in a non-small cell lung cancer cell line selected for doxorubicin resistance that did not express P-glycoprotein (Scheper et al., 1993). In addition to the ABC transporters, LRP has been implicated in drug resistance, being a superior predictor for in vitro drug resistance compared with P-glycoprotein (Kitazono et al., 1999). Initially, this protein was named the lung resistance protein, but later was identified to be the human major vault protein (MVP) (Scheffer et al., 1995). LRP mRNA levels have been associated with the multidrug resistance phenotype in various tumors providing evidence for its role in the multidrug resistance (Ferguson et al, 2005; Laurencot et al, 1997; Wang et al., 2004). Vaults are ribonucleoprotein particles found in the cytoplasm of eukaryotic cells, which consist of 96 copies of MVP, two telomerase-associated proteins (TERP1) and eight molecules poly-ADP ribose polymerases (VPARP) and at least three copies of untranslated RNA, namely hvg 1, 2 and 3 (Kong et al., 2000; Zheng et al., 2005). Vaults appear to be hollow barrel-like structures with 8-2-2 symmetry. Each has an invaginated waist and two protruding caps. They can fall apart in two parts that can unfold in flower-like structures. Although vault function is undetermined, it has been proposed that vaults may mediate transport of various substances. A role for vaults in intracellular traffic might be mediated by binding to cellular organelles through direct interaction with its targets. The majority of vault particles are distributed throughout the cytosol, but a portion has been localized to the nuclear membrane at or near the nuclear pore complex, which may prove the role of vaults in the nucleo-cytoplasmic exchange (Schroijers et al., 2000). Based on evidence with fluorescent anthracyclines in LRP expressing cell lines, it was proposed that vaults

could act by extrusion of the drugs from the nucleus and sequestration into vesicles (Ferguson et al., 2005), but the later fate of those vesicles remains unclear. Possibly the vaults are transformed into exocytotic vesicles and extrude xenobiotics by means of exocytosis, but they also could act indirectly in cooperation with ABC transporters such as P-glycoprotein and MRP1 (Fig.1.4.3.1) (Mossink et al., 2003). Recent studies reported that doxorubicin upregulated vaults and P-glycoprotein independent resistance were acquired (Cheng et al., 2000). In non-small cell lung cancer cells, LRP expression levels, determined on mRNA and protein level correlated with resistance to cisplatin (Berger et al., 2005). Moreover, LRP expression closely reflected known chemoresistance characteristics. Clinicopathology studies showed that LRP expression, rather than P-glycoprotein and MRP1 expression, is a strong and independent prognostic factor for poor response to chemotherapy in ovarian carcinoma and leukemia (Izquierdo et al., 1998). Using a LRP induction system and LRP- specific ribozymes was demonstrated that LRP is involved in resistance to doxorubicin, cisplatin, vincristine, VP-16, taxols and grandamycin D and is of a vital role in the transport of doxorubicin between the nucleus and the cytoplasm in the SW620 human colon cancer cell line (Kitazono et al., 1999). Until now, no specific inhibitor of LRP activity has been described; however, some pyridine analogues known to modulate the activity of MRP1 and P-glycoprotein are also found to modulate the multidrug resistant phenotype in some overexpressing LRP cell lines (Salerno et al., 2004).

Elevated LRP levels were observed in cell lines resistant to various classes of cytotoxic agents including doxorubicin, mithoxantrone, methotrexate, etoposide, vincristine, cytarabin and cisplatin (Berger et al., 2005; Komarov et al., 1998; Wyler et al., 1997).

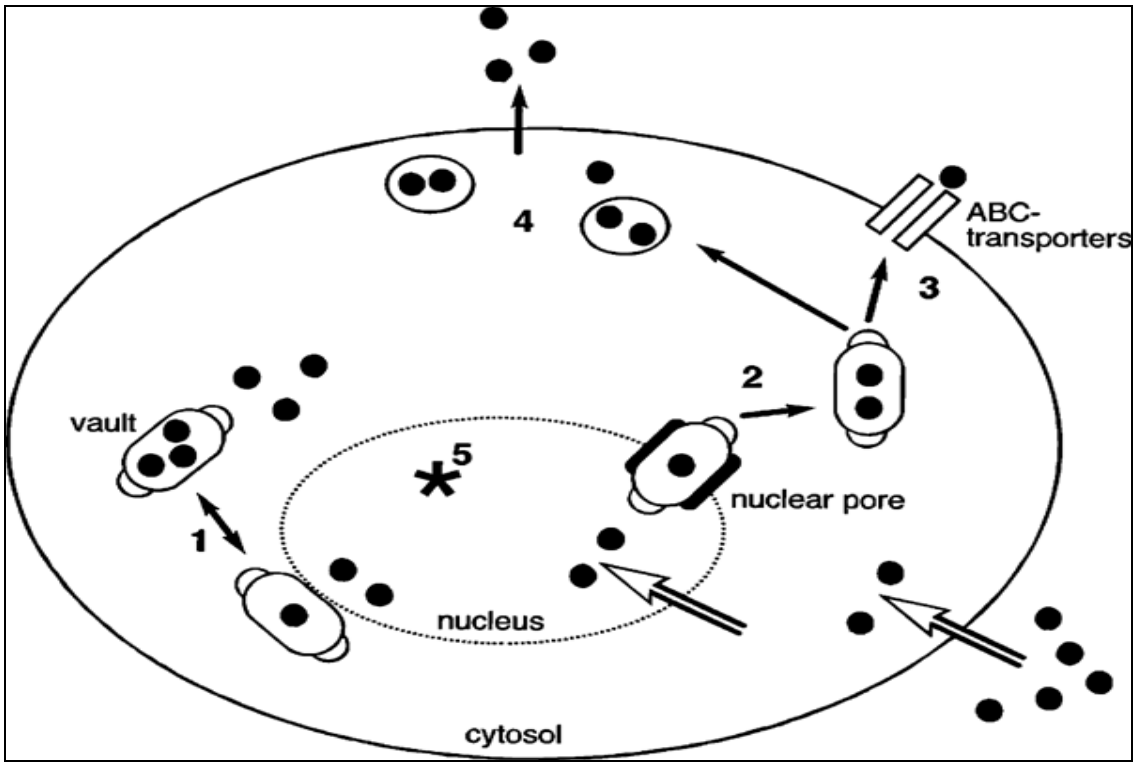

Fig. 1.4.3.1. Possible LRP (MVP) modalities in extruding xenobiotics out of the nucleus (5) - alone by exocytosis (4), in cooperation with an ABC transport protein (3), or by means of redistribution inside the cell (1).

1.5. Modulation of the multidrug resistant phenotype by calcium antagonists

Substantial efforts have been made to identify agents capable of reversing resistance mediated by MRP1 and P-glycoprotein taking into account their clinical relevance. Although many potential modulators have been reported for P-glycoprotein (Table 1.4.1.1), very few have been described for MRP1 (Salerno et al., 2004; Takara et al., 2002). The well-known calcium channel blocker verapamil is a reference for P-glycoprotein inhibition. Moreover, verapamil has been reported to be effective in restoring drug sensitivity in MRP1 overexpressing cell lines. Since the discovery of verapamil's ability to reverse P-glycoprotein -mediated drug resistance, a lot of research work has been devoted to the investigation of the mechanism by which various

modulators inhibit P-glycoprotein function. It was found that verapamil can both stimulate (if the pump is inactive) and inhibit (if actively pumping) the activity of P-glycoprotein, and that verapamil binds reversibly to P-glycoprotein and inhibits the binding of many chemotherapeutic agents as well as other modulators to P-glycoprotein, indicating that verapamil acts through the mechanism of competitive inhibition on P-glycoprotein binding. As non-transported substrates for P-glycoprotein and MRP1 verapamil and its derivates may constitute a candidate for clinical application. Another possible way of its action is by inhibiting the calcium dependent cAMP and cGMP phosphorilazes, which on their behalf regulate the P-glycoprotein activity. Verapamil, however, also blocks MRP1 activity, but at much higher concentrations. As reported by other authors (Takara et al., 2002) 13.5 mM verapamil blocked 50 % of P-glycoprotein mediated drug efflux and 40 mM verapamil blocked 50 % of MRP1-associated drug efflux in a block of cell lines. Although those concentrations are cardiotoxic, new verapamil derivates appear to be promising for the treatment of multidrug resistance in cancer patients. It is poorly understood why some types cell lines, overexpressing P-glycoprotein and MRP1, manage to escape cell death after incubation with cytotoxic drugs, although they are exposed to a concentration of verapamil that blocks the activity of both P-glycoprotein and MRP1. Until now, no specific LRP blockers have been reported, though some LRP substrates could achieve a non-specific LRP blockade due to a substrate high affinity (Kitazono et al. 2001). However, drug resistance in cancer can have a biochemical as well as a physiological basis and it is not certain that reversal of drug resistance in a biochemical sense could result in a cure.

1.6. Therapy modalities in the treatment of breast and colorectal cancer

Breast and colon cancers are known to possess a high relapse risk and therefore adjuvant chemotherapy and radiotherapy are of enormous significance (Wolmark et al., 2000). Adjuvant radiotherapy in breast cancer is obligatory in most patients. The cancer focus usually undergoes 50 Gy of irradiation treatment, which is divided into 1.8 – 2 Gy daily doses. Additionally, a boost of 10 - 16 Gy is given to the logé; in case of lymph nodes

involvement, the axillary's region is also irradiated. The combined approach is also an option in rectal cancer, where the small pelvis could be irradiated with total doses of 45-55 Gy, using 1.8 - 2 Gy daily doses. There is no standard for chemotherapy of breast cancer; however, in adjuvant as well as in palliative chemotherapy of breast and colorectal cancers, alkylating agents and topoisomerase inhibitors are commonly included in the therapy schemes (Adlard et al., 2002; Goldhirsch et al., 2001; Schrag et al., 2001). Recently, monotherapy schemes with bendamustine (a novel alkylating agent) and anthracyclines (topoisomerase inhibitors) have also been investigated in the treatment of advanced breast cancer.

1.7. Thesis

Drug resistance occurring after radiotherapy is a major problem that limits the effectiveness of chemotherapy and is often accompanied by P-glycoprotein and MRP1 overexpression. On the other hand, it is known that drug resistance is often due to overexpression of these two ABC transporters and LRP, the human major vault protein. The latter is represented as the best individual predictor of drug sensitivity in vitro.

The observed earlier P-glycoprotein and MRP1 overexpression occurring after gamma-irradiation is systematically evaluated and elaborated, using a fractionated protocol reflecting the clinical situation. In addition, the LRP expression is determined. Chemosensitivity assays are carried out with chemotherapeutics frequently used in practice, namely – cisplatin and doxorubicin and the novel drug bendamustine. An objective of the present investigation is to explore a potential correlation between the level of drug resistance after radiation therapy and the overexpression of multidrug resistance associated proteins.

The present investigation aims to answer the question whether overexpression of P-glycoprotein, MRP1 and LPR would be accompanied by functional drug resistance to bendamustine cisplatin and doxorubicin, but also if the cell resistance following irradiation is modulated by a potent P-glycoprotein blocker.