Circumvention and/or inhibition of active efflux transporters in cancer and brain diseases.

Multidrug resistance in cancer or inefficacy of neuroactive agents: innovative strategies to inhibit or circumvent the active efflux transporters selectively

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Multidrug resistance (MDR) is a crucial issue in the treatment of cancer cells that protect themselves by overexpression of active efflux transporters (AETs). AET expression maintains the homeostasis in healthy tissues and in the blood–brain barrier it often prevents drugs from reaching the brain. Inhibition of AETs could therefore be a valuable solution for preventing MDR; but nonselective long-term AET blocking can be harmful toward healthy tissues and, in particular, the brain. This review looks at the development of innovative formulations suitable for selectively blocking or avoiding AETs as promising ways to overcome the challenges of MDR and inefficacy of neuroactive agents.

Introduction

The number of people with cancer or brain diseases is currently high [1–5], even if enormous progress toward the comprehension of the pathogenesis of these diseases has been made. The difficulties that arise for therapies against cancer and brain diseases appear essentially to be related to the multidrug resistance (MDR) phenomenon and to the physiological barrier protecting the brain. The current review addresses one of the most important issues related to the therapies against cancer and brain diseases [*i.e.* the active efflux transporters (AETs)]. Indeed, the first defense of cells against xenobiotics is the AET expression on their membrane, with consequent reduction of intracellular drug concentration in normal and cancer cells. It is hypothesized that several AETs become upregulated in some cancer cells during chemotherapy or that a small percentage of cancer cells have intrinsically higher levels of several AET types, allowing them to survive and, thus, induce MDR [6]. Moreover, brain homeostasis is ensured by the blood–brain barrier (BBB) and the blood–cerebrospinal-fluid barrier (BCSFB), which separate the brain from the blood supply, controlling the entry and the exit of endogenous and exogenous compounds [7,8]. The permeation of molecules across the BBB and BCSFB is severely restricted by the presence of AET systems, allowing neuroprotection from harmful molecules. The permeation of small and large molecules across these physiological barriers appears therefore to be the exception rather than the rule [4,5].

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TABLE 1

Main ABC transporters responsible for multidrug resistance in cancer

Transporter	Name	Gene symbol	Expression	Overexpression in tumors	Anticancer drug substrates
P-glycoprotein	P-gp	ABCB1/MDR1	Placental trophoblasts, testes, intestines, liver, kidneys, adrenal glands	Leukemia, breast, ovarian, colon, kidney, adrenocortical, hepatocellular cancers	Vinca alkaloids (vinblastine, vincristine, vindesine, vinorelbine), anthracyclines (doxorubicin, daunorubicin), taxanes (paclitaxel, docetaxel), methotrexate, mitoxantrone, imatinib mesylate, saquinavir
Multidrug Resistance Protein	MRP1	ABCC1/MRP1	Basolateral membrane in polarized epithelial cells	Lung, breast, prostate, ovarian cancers, gastrointestinal carcinoma, melanoma, leukemia	Vinca alkaloids, anthracyclines, methotrexate, mitoxantrone
Breast Cancer Resistance Protein	BCRP	ABCG2/BCRP	Placenta, brain, liver prostate, intestine, stem cells	Leukemia, breast cancer	Mitoxantrone, topotecan, methotrexate, docetaxel, paclitaxel, saquinavir, flavopiridol

Innovative drugs, prodrugs or formulations are therefore required to evade AET systems when neuroactive agents need to target the brain. Indeed, these innovative systems should enable drugs to target the central nervous system (CNS), without altering AET-regulated brain homeostasis. By contrast, innovative drugs, prodrugs or formulations are also needed to target anticancer agents and AET inhibitors in specific tumors. In this case, the concomitant presence of AET inhibitors and anticancer agents in tumoral tissues should induce a 'killer' synergic effect against cancer cells; this effect being focused only where it is necessary in the body should reduce the unwanted effects related to chemotherapy. The strategies aimed to design these innovative therapeutic systems will be described.

AETs: is their inhibition a solution against MDR or poor brain permeability of neuroactive agents?

AETs are normally expressed in healthy tissues, including liver, placenta, the proximal tubule in the kidney, endothelial cells of brain capillary, testis and enterocytes [9,10], where they act as a part of the detoxification systems and contribute to regulate, in normal physiologic conditions, the absorption, distribution, metabolism and elimination of endogenous and exogenous substances [11]. The AET systems can be members of two transporter gene superfamilies [4,5,12]:

i The ATP-binding cassette (ABC) gene family – in this case the AET systems are energy-dependent primary active transporters coupling ATP hydrolysis to active efflux of their substrates against a concentration gradient.

ii The solute carrier (SLC) gene family – in this case the AET systems are energy-independent passive or secondary active transporters.

It is believed that coordination between ABC and SLC transporters allows efficient vector-mediated transport across the BBB and BCSFB cells to remove xenobiotics from the brain [13–15]. Table 1 summarizes the ABC transporters considered the major players in the development of MDR in cancer, reporting also the physiologic expression of these transporters in healthy tissues, the tumors where they are overexpressed and the anticancer drugs identified as their substrates [14,16,17]. Table 2 summarizes membrane transporters mainly involved in drug delivery against brain diseases, indicating by which brain defense barriers they are expressed, together with a list of brain tumors where they are overexpressed and anticancer drugs identified as their substrates [15,18].

Tables 1 and 2 clearly designate ABC and SLC transporters as mainly responsible for the MDR connected with cancer diseases and poor brain permeability of neuroactive agents. The inhibition of these efflux systems could apparently be a valuable solution to prevent MDR in cancer, but it is important to remark that a nonselective long-term blocking of AET systems can be harmful toward healthy tissues and especially toward the CNS, where the AETs, expressed on the BBB and BCSFB, maintain the homeostasis. Therefore, an innovative strategy to overcome the problems related to MDR and poor brain permeability of neuroactive agents should take into account two important aspects: (i) tumors require a strategy so that they can selectively target AET inhibitors and anticancer agents in their action site; (ii) brain diseases require a strategy allowing the neuroactive agents to circumvent the AET systems. In this regard, it is interesting to note that the latest exploratory studies support the possibility of a true inverse relation between cancer and neurodegenerative disorders, such as Alzheimer's disease [19]. Amyloid Aβ peptides, cut from the amyloid precursor protein of neurons, have been assumed to induce wide neuronal apoptosis in the brain, as shown by *in vitro* experiments using established neuronal cell lines [20]. However, the increased ABC transporter activity at the BBB of brain tumor survivors enhanced the extrusion of these amyloid Aβ peptides from Alzheimer's-disease-relevant sites, stopping their apoptotic effect [20]. Clearly, further work is required to establish the link between these two groups of brain diseases better, but this emerging evidence is another instance where therapeutic strategies that bypass without inhibiting ABC efflux pumps in the CNS are needed [21,22].

Finally, it is interesting to remark that the strategy to block the AET systems in tumor cells selectively is corroborated by the evidence that the forced accumulation of intracellular cyclic

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