

The Use of Anthracyclines for Therapy of CNS Tumors

Martina da Ros, Anna Lisa Iorio, Maurizio Lucchesi, Alessia Stival, Maurizio de Martino and Iacopo Sardi*

Neuro-Oncology Unit, Department of Paediatric Medicine, Anna Meyer Children's University Hospital, Florence, Italy

Abstract: Despite being long lived, anthracyclines remain the “evergreen” drugs in clinical practice of oncology, showing a potent effect in inhibiting cell growth in many types of tumors, including brain neoplasms. Unfortunately, they suffer from a poor penetration into the brain when intravenously administered due to multidrug resistance mechanism, which hampers their delivery across the blood brain barrier.

In this paper, we summarize the current literature on the role of anthracyclines in cancer therapy and highlight recent efforts on 1) development of tumor cell resistance to anthracyclines and 2) the new approaches to brain drug delivery across the blood brain barrier.



I. Sardi

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ANTHRACYCLINES IN CANCER THERAPY

Anthracyclines are the class of antitumor drugs with the widest spectrum of activity in oncology clinical practice, as shown in Table 1 [1, 2].

Their chemical structure is formed by a tetracyclic ring with quinone-hydroquinone adjacent groups [3]; the antitumor activity is related to topoisomerase II inhibition, which is due to the anthracyclines intercalation between double strand DNA. An interesting study by Frederick *et al.* very clearly helps to perceive how DOX and DNR are inserted between DNA base pairs [4].

However, the molecular mechanism(s) by which anthracyclines cause cell death or cardiotoxicity remains unclear. A number of models have been proposed for anthracyclines-mediated cell death, including topoisomerase II poisoning, DNA adduct formation, oxidative stress and ceramide overproduction, but most of them remain disparate and controversial. Recently, Yang and co-workers have reviewed the effects of anthracyclines on DNA torsion and chromatin dynamics; the authors suggested that combining anthracyclines and other drugs that destabilize nucleosomes may have synergistic effects in killing cancer cells [5].

DOX remains the most widely administered of the anthracyclines [1, 2], although its clinical application is limited by associated toxicities, including myelosuppression, nausea, vomiting, stomatitis, alopecia and, most importantly, cardiotoxicity [1].

In addition to problems with cardiotoxicity, anthracyclines suffer from poor efficacy due to multidrug resistance (MDR), which hampers their delivery across the blood brain barrier (BBB).

At the present, various strategies have been emerged in order to overcome the MDR mechanism(s).

Anthracyclines in Central Nervous System (CNS) Tumor Therapy

Among the variety of anticancer drugs, anthracyclines show a potent effect in inhibiting cell growth in many types of tumors, including CNS neoplasms [1, 2]. On the basis of *in vivo* and *in vitro* evidence, brain tumors are expected to be sensitive to chemotherapy

agents, as anthracyclines [6-8]. Yet, most patients fail to achieve an adequate disease control due to a limited CNS drug penetration, as Von Holst and co-workers have demonstrated in patients with malignant gliomas [9].

A wide range of factors influence the drug uptake into the brain. One of the underlying mechanisms of multidrug resistance is the over-expression of influx and efflux proteins that work *in tandem* on the BBB [10].

MDR PHENOTYPE

The Multi Drug Resistance (MDR) is a complex biological phenomenon. Proposed mechanisms underlying MDR at the cellular level include increased drug efflux, decreased drug activation, genetic alterations that affect different aspects of cellular physiology, DNA methylation, induction of DNA repair pathways, inhibition of apoptosis and, specially, elevated expression levels of drug efflux pumps [11, 12].

The resistance can be innate or acquired. It may exist in tumor cells since the beginning of therapy or tumor cells may acquire resistance during treatment, becoming cross-resistant to a range of chemically unrelated compounds.

Cross resistance is primarily due to an overexpression of drug transporters on cancer cell membrane. These transporters, known as MDR proteins, are able to act against a wide range of different anticancer agents, including drugs which have not yet been administered to the patient [12].

Both prokaryotes and eukaryotes express MDR proteins; in humans, they are physiologically located in many tissues and organs (bronchial or gastrointestinal tract, brain endothelium, breast, liver, kidneys, testis), both on plasma membrane and intracellular membrane, working as “gatekeepers”. Their main physiological role is to defend the organism from the deleterious effects of xenobiotics and/or endogenous toxic.

Unfortunately, this mechanism is amplified in patients undergoing chemotherapy treatment: cancer cells can constitutively over-express these transporters to increase the drug efflux (for example cells derived from glioblastomas) [13]. Increases in drug efflux are responsible for enhanced drug resistance.

Since not only a single drug, but also chemically unrelated compounds can be involved, we refer to it as Multi Drug Resistance. Furthermore, it has been demonstrated that tumors evolved from cells where MDR proteins are physiologically absent

*Address correspondence to this author at the Neuro-Oncology Unit, Department of Paediatric Medicine, Meyer Children's Hospital. Viale G. Pieraccini 24, 50139 Florence, Italy; Tel: +39 055 5662631; Fax: +39 055 5662746; E-mail: iacopo.sardi@meyer.it

Table 1. Solid tumors and hematological malignancies for which anthracyclines are effective antineoplastic drugs.

Doxorubicin (DOX)	Daunorubicin (DNR)	Epirubicin (EPI)	Idarubicin (IDA)
Breast Cancer Ovarian Cancer Transitional cell bladder cancer Bronchogenic lung cancer Gastric cancer Thyroid cancer Osteogenic sarcomas Soft tissue sarcoma Neuroblastoma Wilms' tumor Hodgkin and non-Hodgkin lymphoma Acute myeloblastic leukemia Acute lymphoblastic leukemia Kaposi's sarcoma	Acute myeloid leukemia Lymphatic leukemia	Ovarian cancer Breast cancer Small cell lung cancer Rectal cancer Gastric cancer Pancreatic cancer Soft tissue sarcoma	Acute myeloid leukemia

Doxorubicin (DOX), Daunorubicin (DNR), Epirubicin (EPI) and Idarubicin (IDA) are the primary anthracyclines for therapy of many types of cancer.

can exhibit expression and functional activity of these transporters. Gliomas are an example [13].

Among the drugs that are most frequently associated with MDR are counted the anthracyclines [14-16]. The action of MDR on anthracyclines is complex and not fully understood. It primarily based on the over-expression of ATP-binding cassette (ABC) proteins, as P-glycoprotein (ABC1/P-gp/MDR1), multidrug resistance protein (MRP/ABCC) family and breast cancer resistance protein (BCRP/ABCG2), all of which drive efflux away from the CNS by an ATP-dependent process.

P-gp and BCRP are the most studied MDR proteins. *In vivo* evidence shows that their expression and activity can be considered as a marker for chemotherapy resistance and prognosis in patients with ovarian cancer and acute myeloid leukemia [17, 18].

If the over-expression of efflux pumps is the major determinant of chemoresistance at cellular level, the low efficacy of chemotherapy in CNS tumor is primarily due to the Blood Brain Barrier (BBB).

In this review we focused on the MDR mechanism P-gp/BCRP mediated at the BBB level.

MDR at the Blood-Brain Barrier

BBB is an anatomic and metabolic barrier between blood and brain, which controls and limits the spread of toxic insults within the brain, thus representing a major physical and physiological hurdle for the drug delivery to the brain.

The endothelial cells forming the BBB are its mainstay, along with astrocyte, pericyte, and the adjacent neurons.

These cells differ from endothelial cells from other organs for the lack of fenestration, few pinocytotic vesicles and tight junctions that are responsible for a restricted cell permeability, by limiting the movement of molecules from the blood to the brain. Under normal physiological conditions, the brain capillary endothelium functions to hinder the delivery of many potentially toxic agents to the brain, including chemotherapy agents. It achieves this goal by efflux transporters and tight junctions [19].

On the basis of their molecular weight and lipophilicity, molecules cross the BBB to reach the tumor site at a therapeutic concentration. A normal BBB prevents the passage of agents with molecular weight >400-600 Da and only those that are highly lipid soluble are able to cross it [20].

However, there are exceptions and, in many cases, the low drug delivery across the BBB can be ascribed to the mechanism(s)

of unidirectional efflux mediated by ATP-dependent transporters [21].

The ABC pumps are expressed at the apical side of the BBB; among them, P-gp is the main "gatekeeper" explored. P-gp substrates, flowing into the endothelium, are rapidly pumped out of the cells, and thus not allowed to enter the brain. Consequently, their penetration from the blood into the brain parenchyma is considerably decreased. Indeed, it has been reported that blocking P-gp by specific inhibitors significantly increases the brain concentrations of antiepileptic drugs [22]. Recently, Abdallah and co-workers have reviewed the role of P-gp inhibitors as potential tumor chemo-sensitizers [23].

Many chemotherapy agents are substrates of P-gp, BCRP and MRPs, whose ATP-dependent active transport at the BBB is considered one of the most relevant mechanisms of tumor resistance [21].

In vitro studies showed that anthracyclines seem to be active drugs on gliomas and other brain tumors [24]. Nevertheless, this effect lacks *in vivo*. It at least in part explains the inability to deliver therapeutic agents to the CNS across the BBB due to drug efflux mechanism(s) [25,26].

New experimental approaches are therefore needed to overcome MDR at the BBB.

In addition to invasive, pharmacological and physiological methods [27], another interesting way to enhance brain cancer therapy is through the use of nanoparticles (NPs) [28].

Here we focus on the specific nano-delivery systems as a non-invasive technique of anthracyclines delivery into the brain.

ANTHRACYCLINE DELIVERY SYSTEMS TO OVERCOME MULTIDRUG RESISTANCE AT THE BLOOD BRAIN BARRIER

Over the years, many different strategies have been employed to overcome MDR at the BBB; a number of studies regarding the treatment of brain diseases have demonstrated the key role of specific drug carriers [29].

Current drug delivery systems that show great promise and are considered to be more popular for clinical applications include liposomes, polymer and peptide/protein conjugates, polymeric micelles, polymeric, lipid and inorganic nanoparticles [16, 28, 30]. In order to find an effective brain drug delivery system, liposomes and nanoparticles have been extensively used because a wide range of molecules can be easily incorporated into their construct [31].

In a recent report, Masserini described the factors affecting nanoparticles brain drug delivery and the mechanisms by which nanoparticles can cross the BBB [32].

Liposome

Liposomes are small artificial vesicles consisting of lipid bilayers, capable of packaging drugs for many delivery applications. The first liposomal formulation to achieve approval in oncology clinical practice was doxorubicin HCl liposomal injection (Doxil) [33], followed by pegylated liposomal doxorubicin (Doxil/Caelyx [PLD]), non pegylated liposomal doxorubicin (Myocet [NPLD]), liposomal daunorubicin (DaunoXome) and liposomal cytarabine (DepoCyte) [34, 35].

Liposomes have been extensively studied as an effective system for brain drug delivery [36] and treatment of CNS tumors [37]; literature data suggest that liposomal formulation exhibits a decreased toxicity and a better tolerability compared to free drugs [38]. Moreover, it not only has activity against tumor types with known sensitivity to conventional anthracyclines, but also potentially for tumors that are typically anthracycline-resistant [39, 40] and it able to reverse drug-resistance by inhibiting P-gp in human cancer cells [41].

Specially PLD is considered an effective class of DOX delivery system. A number of preclinical tumor models has been performed to evaluate its effectiveness in inhibiting or halting tumor growth [42], demonstrating that liposomal encapsulation significantly improved the penetration of DOX across the BBB compared to free DOX [43, 44]. Furthermore, it was able to induce remission in CNS tumors and partial reversal of drug resistance in cell cultures [45].

Nevertheless, slow release, poor availability of the incorporated drug or low physical stability can hamper the clinical applications of liposomes [46,47]. Recently, solid lipid nanoparticles (SLNPs) have been shown to enable CNS drug delivery, representing useful alternative delivery systems to liposomes [48]. A very interesting *in vitro* study has been performed by Battaglia's group who has demonstrated that DOX-loaded SLN can be successfully exploited for the glioblastoma treatment [49].

Polymer and Peptide/Protein Conjugates

Kopeček *et al.* performed the synthesis of a conjugate of N-(2-hydroxypropyl) methacrylamide (HPMA) and DOX [50]. Since then, the HPMA-DOX conjugate has been well studied. A number of experiments demonstrated the promising future of this conjugate which is able to increase cell apoptosis, lipid peroxidation and DNA damage compared to free DOX, to overcome *MDR1*, to down-regulate *MRP*, to prevent *MDR de novo* development and to inhibit the intracellular repair mechanisms [51-54].

Over the years, several DOX polymer conjugate (DOX-dendrimer conjugate or dextran-DOX conjugate) and DOX peptide conjugates (maurocalcine, penetratin, TAT, transferrin-DOX conjugate, bovine serum albumin (BSA)-DOX conjugate) have been synthesized to increase the antitumor efficacy in resistant cells. Despite the mechanism by which polymer and peptide/protein conjugates can cross the cell membrane remains unclear, many preclinical models have been developed to identify a more effective BBB penetrating polymer and peptide [55].

Polymeric Micelles

Polymeric micelles are colloidal carriers characterized by a spherical core-shell structure which loads the drugs. One of the most utilized polymers is pluronic type, composed of poly(ethyleneoxide)-block-poly(propyleneoxide)-block-poly(ethyleneoxide) [32].

Based on the results from the drug copolymer binding studies, a number of micelle formulations has been developed. They include DOX-encapsulated poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEOPPO-PEO) micelles [56], copolymers containing poly(L-aminoacid) and poly(ester) hydrophobic blocks [54], poly(L-lactide)-vitamin E TPGS (PLA-TPGS) block copolymer [57]. All of them seem to be effective carriers of many therapeutic agents to the CNS, including DOX [16, 58].

Polymeric Nanoparticles

Polymeric nanoparticles (NPs) are colloidal carriers characterized by a core polymer matrix (natural or synthetic), a high physical stability, simple formulations/procedures for preparation and, specially, rapid biodegradability [55].

NPs can be attached to many types of small ligands, including chemotherapy drugs, to be then transported across the BBB *via* either receptor-mediated transcytosis, or adsorptive-mediated transcytosis. The properties of enhanced permeability and retention effect allow their accumulation in brain tissue.

Poly(butylcyanoacrylate) (PBCA), polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides, polycyanoacrylates and polycaprolactone nanoparticles are the most popular synthetic polymer-based nanoparticle systems [32, 59]. Recently, Kreuter has well reviewed the mechanism of drug delivery to the CNS by NPs, highlighting that PBCA, PLA, PLGA are the most suitable particles for the uptake of drugs into the brain [60, 61].

Their surface can be coated with hydrophilic surfactants, such as polysorbate 80 or poloxamer 188, and tested for drug delivery to the CNS, including DOX. Noteworthy, it has been demonstrated that the surface properties of these particles, i.e. the surfactants used as coated agents, are responsible for the delivery of the loaded drugs [60].

A number of literature data underlined the use of polymer-based NPs with DOX as a promising drug delivery system across the BBB [61-65]. Scientific evidence have demonstrated that the nanoparticulate formulation of DOX (poly(butyl cyanoacrylate (PBCA) or poly(lactic-co-glycolic acid) (PLGA) coated with polysorbate 80 or poloxamer 188 enabled a considerable tumor growth reduction in rat glioblastoma, without increased toxicity [66, 67]. This formulation not only increased the survival times and the antitumor effect of the drug, but showed a higher cytotoxicity than free DOX both *in vitro* and *in vivo* tumor models [68, 69].

Moreover, interesting studies have been performed to search both natural and synthetic substrates able to induce the brain targeting of NPs.

Many types of natural compounds have been reported as targeting ligands: transferrin and/or insulin, or antitransferrin and/or anti-insulin-receptor monoclonal antibodies can be conjugated to the surface of NPs to enable their brain targeting [70].

Recently, Xin and co-workers have successfully explored the effectiveness of a synthetic peptide, known as angioprep nanoparticle (ANG-NP) for targeting therapy of brain tumors [71]. ANG-NPs showed a high efficiency in crossing BBB by targeting the low-density lipoprotein receptor-related protein (LRP) which is over-expressed on the BBB and glioma cells. As a result, ANG-NPs can specifically deliver and release the loaded drugs, as DOX, in tumor cells [72].

Various Angioprep vectors have been composed. They appear to be a promising choice for cancer therapy that can not only be related to anticancer agents but also peptides, monoclonal antibodies or siRNA [70].

Finally, recent studies have demonstrated the effectiveness of NPs loaded with efflux pump inhibitors and chemotherapeutics both *in vitro* and *in vivo* models [59, 70].

Inorganic Nanoparticles

Other popular carriers explored for drug delivery are the inorganic NPs [73], which include magnetic Fe₃O₄ NPs, gadolinium NPs, gold NPs (AuNPs) and semiconductor quantum dots [28].

A promising nanomaterial for the therapy of malignant brain tumors is represented by magnetic NPs (MNPs) and iron-oxide nanoparticles (IONPs) [74].

Among the biomedical applications, MNPs can be utilized to deliver chemotherapeutic agents to the brain tumor site. IONPs coated with a polymer have been used for both delivery of the chemotherapy agent epirubicin and monitoring of their distribution *in vivo* by MRI [74,75]. Liu *et al.* have demonstrated the synergistic effect of focal ultrasound and magnetic targeting in a study where both systemic delivery and deposition of epirubicin-loaded MNPs into tumor-bearing animals were found to be significantly increased [75].

Literature data underlined that gold NPs and nanodiamonds are other highly efficient carriers of many drugs, including DOX, for the treatment of malignant gliomas [76, 77]. In fact, these formulations are able to kill tumor cells more effectively than DOX alone, paving the way for their use in the treatment of chemoresistance brain tumor [76, 77].

In conclusion, NPs technology may represent a clinical concrete reality for the non-invasive chemotherapy of CNS diseases.

REVERSIBLE BBB ALTERATION BY PHARMACOLOGICAL APPROACH

The cure rate of malignant brain tumors lags behind the success obtained in other areas of hematology-oncology. This depends also on limited diffusion of chemotherapy agents within the brain tissue. In a recent report, Sharma and co-workers demonstrated that morphine induces a reversible alteration of BBB permeability to large molecules in the rat [78].

The question is whether it is possible to hypothesize that morphine or other agents may induce a reversible modulation of the BBB, with the aim to allow controlled permeability to chemotherapy drugs.

In order to find a safe and reliable way to deliver anticancer agents within the brain for the treatment of brain tumors, our group has recently investigated the possibility to permeabilize BBB by using morphine for delivery of DOX into the rat brain [79, 80].

Our experiments have demonstrated that morphine (10 mg/kg, *i.p.* three times in 24 h) allows an accumulation of DOX (12 mg/kg, *i.p.* 1 h after the last injection of morphine) within the rat brain by LC-MS/MS mass spectrometry [79]. DOX concentration was significantly higher in all brain areas of animals pretreated with morphine in comparison to the group of animals treated with DOX alone: + 254% *vs* DOX alone in the cerebral hemispheres; + 308% *vs* DOX alone in the cerebellum; + 256% *vs* DOX alone in the brainstem. The data indicate that morphine at therapeutic dose of 10 mg/kg, *i.p.*, three times in 24 h, facilitates the entrance and/or the accumulation of the anthracycline into the brain tissue. A lower concentration of morphine (5 or 2.5 mg/kg, three times in 24 h, *i.p.*) was not effective for inducing DOX entry into the brain.

Moreover, since DOX is a cardiotoxic drug and its use can induce iatrogenic life-threatening cardiomyopathy and kidney toxicity, we quantified the DOX level in these tissues after morphine pre-treatment. No differences were found between rats treated with DOX alone and rats also treated with morphine,

indicating that pre-treatment with morphine might be effective to increase brain levels of DOX, without anthracycline-associated cardiac and renal toxicity. Acute hearth and kidney damage was also analyzed by measuring LDH activity and MDA plasma levels: pre-treatment with morphine did not increase LDH activity or lipid peroxidation compared to controls [79]. Finally, we did not observe any clinical toxicity in rats pre-treated with morphine compared to control group 10 days after treatment, indicating that the co-administration of morphine does not cause late neurological deficit [79].

The mechanisms underlying transient alteration of the permeability of BBB are not yet known. It is noteworthy that morphine and DOX are substrates of P-gp/BCRP efflux pump [26, 81]. Therefore, we hypothesize that morphine or other agents can act as an agonist of DOX efflux mediated by P-gp and BCRP, localized in the BBB, increasing the access of drug in tumor cells.

In this regard, we have recently performed a study to assess whether ondansetron (Ond) enhances DOX cytotoxicity in glioblastoma cell lines interfering with P-gp and it is able to increase DOX concentration in rat brain tissue [82]. Ond is commonly used during chemotherapy as a potent antiemetic drug and it is a specific substrate of P-gp efflux pump [26].

Our *in vivo* data showed that Ond (2 mg/kg, *i.p.* three times in 24 h) is an effective "door-keeper" in allowing accumulation of DOX (12 mg/kg, *i.p.* administered 1, 1.30 or 2 h after the last injection of Ond) within the brain, as well as morphine. DOX concentration was higher in all brain areas of animals pretreated with Ond in comparison to the group of animals treated with DOX alone, as following: in the brainstem + 67% *vs* DOX alone at 1 h, + 106%, *vs* DOX alone at 1.30 h, + 180% *vs* DOX alone at 2 h; in the cerebral hemispheres + 90% *vs* DOX alone at 1 h, + 130%, *vs* DOX alone at 1.30 h, + 250% *vs* DOX alone at 2h; in the cerebellum + 67% *vs* DOX alone at 1 h, + 117%, *vs* DOX alone at 1.30 h, + 133% *vs* DOX alone at 2h. The data at 2h was statistically significant (one-way ANOVA Dunnett's Multiple Comparison Test, $P < 0.05$). No increased cardiac and renal toxicity and no difference in LDH activity and MDA plasma levels were found between rats treated with DOX alone and those receiving Ond *plus* DOX ($P > 0.05$, Student's *t*-test).

In addition, we have performed *in vitro* experiments in order to explore the ability of Ond to modulate the MDR phenotype in cancer (P5-derived MDR1 cells P1(0.5) hepatocellular carcinoma (HCC) cell lines) and not cancer cells (mdr1-transfected (PN1A) NIH/3T3 cells) that express P-gp. We also evaluated the effectiveness of the Ond (10 and 30 $\mu\text{g/ml}$) exposure and the co-treatment Ond (10 and 30 $\mu\text{g/ml}$)/ DOX (0.1 $\mu\text{g/ml}$) on killing cancer cells, by using different cell lines (human hepatocellular carcinoma drug sensitive cell line PLC/PRF/5, clone (P5); P1(0.5) clone; PN1A clone; drug-sensitive (PSI-2) NIH/3T3 cells; U87MG and A172 glioblastoma cell lines).

Our data demonstrated the ability of Ond (10 and 30 $\mu\text{g/ml}$) to reverse the MDR phenotype in P-gp positive cells which exhibited a decreased survival when treated with Ond (10 and 30 $\mu\text{g/ml}$) *plus* DOX (0.1 $\mu\text{g/ml}$). Conversely, no effect has been observed in PSI-2, U87MG and A172 cells, all of which do not express P-gp and remain sensitive to the killing effect of DOX [82].

Are not yet known the mechanisms underlying transient alteration of the permeability of BBB. It is therefore conceivable that morphine and/or Ond can act as an agonist of DOX efflux mediated by P-gp localized in the BBB, increasing access of the drug in tumor cells [81]. Using them, we can induce a reversible modulation of the BBB, and then open the door to new avenues for the delivery of other macromolecules into the brain, potentially increasing the survival rate of patients with CNS tumors but also with debilitating neurological disorders.

CONCLUSION

Several studies underline that anthracyclines are effective chemotherapeutics for the treatment of gliomas and other CNS tumor types. Unfortunately, this effect lacks *in vivo*.

A prerequisite for the efficacy of chemotherapy is that it reaches the tumor mass at a therapeutic concentration.

In CNS tumors this phenomenon is hampered by the presence of the BBB, a physical and metabolic barrier between blood and brain, which maintains homeostasis and protects the brain from toxic insults, thus representing a major physical and physiological hurdle for the delivery of chemotherapy agents into the brain.

It is lately emerged as this MDR phenomenon is explained through the cooperation of P-gp, BCRP, and MRP proteins, "gatekeeper" transporters that work *in tandem* on the BBB and are present on the plasma membrane of certain brain tumors. Although several alternative strategies have been proposed to increase drug delivery into brain parenchyma, most of these are invasive surgical procedures with high risk of adverse effects, including seizures and hemorrhagic events.

As exemplified by the many studies summarized in this review, the use of nanomedicine seems to be a great promise for anthracyclines delivery, as a non-invasive therapy of multidrug resistant CNS malignancies.

A number of studies regarding the treatment of brain diseases have demonstrated the key role of specific natural or synthetic drug carriers, highlighting their ability to enhance the antitumor effect of certain drugs.

Nanoparticles have unique features that make them a promising choice for cancer therapy. These include enhanced permeability across the BBB and retention capacity, ability to load single or multiple anticancer agents on their surface for a rapid delivering into the CNS, preferential accumulation in cancer cells in comparison with normal cells [83], although further studies are need to evaluate their efficacy, safety, and toxicity in humans [84].

In addition, our investigations on preclinical models demonstrated the therapeutic efficacy of pharmacological treatment in malignant brain tumors by safe and temporary BBB permeabilization. Morphine and/or ondansetron pretreatment are able to allow DOX penetration inside the brain by modulating the BBB, potentially enhancing the efficacy of pharmacological treatments of CNS tumors, without increased acute toxicity.

Our working hypothesis is that morphine and/or ondansetron, and possibly other agents known to be substrates of P-gp, may increase safely the brain permeability to DOX, by competing for P-gp mediated transport at BBB level and that, in turn, such phenomenon may improve the efficacy of pharmacologic treatment of malignant gliomas.

On the basis of our preliminary experience and relevant data from *in vitro* and *in vivo* studies, morphine and/or ondansetron and Dox can be used together to treat high grade gliomas. More importantly, their concomitant use may offer a novel approach for a clinical application of active but currently inapplicable drugs in cancer therapy.

Whether this phenomenon may have a therapeutic impact remains to be elucidated.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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