The Veterinary Journal 207 (2016) 63–72

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Review

Mechanisms of tumour resistance against chemotherapeutic agents in veterinary oncology

R. Klopfleisch [a,*], B. Kohn [b], A.D. Gruber [a]

[a] Institute of Veterinary Pathology, Freie Universität Berlin, Robert-von-Ostertag-Straße 15, 14163 Berlin, Germany
[b] Small Animal Clinic, Freie Universität Berlin, Oertzenweg 19 b, 14163 Berlin, Germany

ARTICLE INFO

Article history:
Accepted 30 June 2015

Keywords:
Oncology
Chemotherapy
Resistance
Epigenetics
DNA repair
Cancer stem cells
Epithelial–mesenchymal transition
Apoptosis

ABSTRACT

Several classes of chemotherapy drugs are used as first line or adjuvant treatment of the majority of tumour types in veterinary oncology. However, some types of tumour are intrinsically resistant to several anti-cancer drugs, and others, while initially sensitive, acquire resistance during treatment. Chemotherapy often significantly prolongs survival or disease free interval, but is not curative. The exact mechanisms behind intrinsic and acquired chemotherapy resistance are unknown for most animal tumours, but there is increasing knowledge on the mechanisms of drug resistance in humans and a few reports on molecular changes in resistant canine tumours have emerged. In addition, approaches to overcome or prevent chemotherapy resistance are becoming available in humans and, given the overlaps in molecular alterations between human and animal tumours, these may also be relevant in veterinary oncology.

This review provides an overview of the current state of research on general chemotherapy resistance mechanisms, including drug efflux, DNA repair, apoptosis evasion and tumour stem cells. The known resistance mechanisms in animal tumours and the potential of these findings for improving treatment efficacy in veterinary oncology are also explored.

Introduction

Chemotherapy is commonly used to treat cancer in pets, and for some tumour types, such as haematopoietic tumours, chemotherapy is the treatment of choice. Complete remission and disease stability can often be achieved with currently available drugs and protocols. However, in a significant percentage of animals, standard protocols are ineffective due to intrinsic resistance of the tumour against available agents. Furthermore, tumour relapse during or after treatment is observed, commonly or sporadically, due to the development of acquired resistance. The mechanisms of resistance and the therapeutic strategies employed to overcome this resistance are important and topical areas for focus in cancer research.

Chemotherapy research in veterinary oncology is constantly progressing, but many questions on the specific mechanisms of chemoresistance in pets are still unclear. In contrast, numerous studies have been published on the mechanisms of resistance against common anti-cancer drugs in humans and animal models. These studies have shown that the mechanisms of chemoresistance mainly consist of: (1) increased cellular efflux of chemotherapeutic drugs; (2) accelerated drug inactivation or lack of drug activation; (3) changes in drug targets (e.g. mutation and methylation); (4) efficient mechanisms of DNA repair; (5) deregulation of apoptosis; and (6) cancer stem cells as the nucleus of resistance in tumours and epithelial–mesenchymal transition (EMT) (Table 1).

This article provides an overview of the current knowledge of chemotherapeutic resistance and explores the mechanisms that may be relevant and targeted in pet animals with chemotherapy-resistant tumours.

Increased efflux of chemotherapeutic agents from tumour cells

Efflux of drugs from cells is mostly based on membrane transporter proteins. Of these, the ATP-binding cassette (ABC) transporter family is considered to be the most important group of transmembrane proteins which drive the transport of many chemically unrelated drugs across the plasma membrane (Holohan et al., 2013). Of the 49 (or more) members of this family, the multi-drug resistance protein 1 (MDR1, P-glycoprotein [PGP], ABCB1), the MDR-associated protein 1 (MRP1, ABCC1) and the breast cancer resistance protein (BCRP, ABCG2) have been most thoroughly investigated in tumours not only of humans but also of dogs and cats (Fig. 1) (Brenn et al., 2008; Honscha et al., 2009; Gramer et al., 2013; Pawlowski et al., 2013; Choi and Yu, 2014). All three of these proteins, and other members of the family, are capable of eliminating lipids and therefore various classes of hydrophobic chemotherapy drugs, including topoisomerase inhibitors, antimetabolites, platinum compounds and microtubule inhibitors, as well as the tyrosine kinase inhibitors (TKIs) (Holohan et al., 2013).

* Corresponding author. Tel.: +49 30 83862460.
E-mail address: robert.klopfleisch@fu-berlin.de (R. Klopfleisch).

http://dx.doi.org/10.1016/j.tvjl.2015.06.015
1090-0233/

Table 1
Specific mechanisms of resistance to chemotherapeutic agents commonly used in veterinary oncology.

Agent	Target/mechanism	Resistance mechanisms (references)
Alkylating agents		
Cyclophosphamide	DNA-crosslinking, inhibition of DNA repair/ synthesis	Increased efflux (Pawlowski et al., 2013)
Chlorambucil		Increased DNA repair (Fink et al., 1998)
Lomustine		MGMT activity (Sarkaria et al., 2008)
Procarbazine	Unclear, maybe DNA break induction, t-RNA inhibition	
Platinum containing drugs		
Cisplatin	DNA-crosslinking, prevention of DNA-uncoiling/ strand separation	Increased efflux (Pawlowski et al., 2013)
Carboplatin		ERK activity (Mirmohammadsadegh et al., 2007)
		DNA repair (Kwon et al., 2007)
		Replicative bypass (Rabik and Dolan, 2007)
Antimetabolites		
Methotrexate	Dihydrofolate reductase	Melanosomal efflux (Saez-Ayala et al., 2012)
Cytarabine	DNA-synthesis inhibition, DNA-chain termination	Decreased uptake (Cai et al., 2008)
5-Fluorouracil (5-FU)	Thymidylate synthase inhibition, replacement of cytosine, thymidine, uracil in DNA/RNA strands	Decreased activation by DCK, DPD (Cai et al., 2008; Zhang et al., 2008)
		Detoxification by CDD, CDA, NT5C2 (Bardenheuer et al., 2005; Tang et al., 2012)
Gemcitabine	Replaces cytidine DNA-synthesis inhibition	Increased DNA repair (Zhang et al., 2008)
		Induction of anti-apoptotic molecules (Zhang et al., 2008)
		Activation of survival pathways (Arlt et al., 2003)
Topoisomerase I (TOPO1) inhibitors		
Camptothecins (irinotecan, topotecan)	Stabilisation of the cleavable DNA–enzyme complex and thereby inducing DNA damage	TOPO1 mutations (Sugimoto et al., 1990)
		Reduced TOPO expression (Meijer et al., 1992)
		Improved DNA repair (Alagoz et al., 2012)
		Lack of apoptosis induction (Beretta et al., 2013)
		Increase efflux by MDR and BCRP (Chu et al., 1997)
Indenoisoquinoline	Complexing with TOPO1	Unknown so far (Yang et al., 2012)
Topoisomerase II (TOPO2) inhibitors		
Doxorubicin	Inhibition of TOPO2 by DNA intercalation	Increased efflux (Zandvliet et al., 2015)
Mitoxantrone		Amplification of TOPO2 and ERBB2 (Noguchi et al., 2014)
Microtubule poisons		
Paclitaxel	Microtubule stabilisation	Apoptosis inhibition (Mhaidat et al., 2009)
Vinblastine	Microtubule Depolymerisation/stabilisation	Increase efflux (Pawlowski et al., 2013)
Vincristine		Tubulin mutation (Kavallaris et al., 2001)
Vinorelbine		Stathmin, MAP4, y-actin overexpression (Perez, 2009)
Miscellaneous agents		
Prednisone	Unclear, apoptosis induction	Increased efflux (Dhaliwal et al., 2013)
		STAT3, pSTAT3, KIT overexpression (Dhaliwal et al., 2013)
		PTEN loss, AKT1 activity (Piovan et al., 2013)
		Decreased receptor expression (Schlossmacher et al., 2011)
L-Asparaginase	Asparagine deprivation	Increased asparagine synthetase (Chien et al., 2015)
		Decreased cellular efflux of asparagine (Chien et al., 2015)
		Increased L-glutaminase activity (Chien et al., 2015)
		Aspartic acid synthesis (Chien et al., 2015)
		Activation of glutamine uptake (Chien et al., 2015)
Tyrosine kinase inhibitors		
Toceranib	KIT, VEGFR2, PDGFRB	Target mutation (Halsey et al., 2014; Kobayashi et al., 2015)
Imatinib	KIT, PDGFR	Increased MCL1 expression (Amagai et al., 2013)
Masitinib	KIT, PDGFRA	
Genistein	Diverse tyrosine kinases	

CDA, cytidine-deaminase; CDD, cytidine deaminase; DPD, dihydropyrimidine dehydrogenase; ERK, extracellular signal-regulated kinase; DCK, deoxycytidine kinase; HR, homologous recombination; MCL1, myeloid cell leukaemia sequence 1; MMR, mismatch repair; MGMT, O6-methylguanine methyltransferase; NER, nucleotide excision repair; NT5C2, cytoplasmic 5′nucleotidase; PDGFR, platelet-derived growth factor; Replicative bypass, ability of DNA polymerase to bypass DNA cross-links; VGEFR, vascular endothelial growth factor.

MDR1 and resistance

MDR1 is a membrane-bound, ATP-dependent efflux pump that is overexpressed in many human tumours before the start of any chemotherapy and thus contributes to *intrinsic resistance* (Goldstein et al., 1989). MDR1 expression is increased or even induced during chemotherapy in various human tumours and contributes to resistance against platinum-containing compounds, topoisomerase II inhibitors, microtubulin poisons and several TKIs (Table 1) (Holohan et al., 2013).

ABC transporters in animal tumours

MDR1, MRP1 and BCRP expression has been analysed in several canine tumours. In most studies the analysis was restricted to the presence of protein or mRNA, to its correlation with the efficacy and outcome of the chemotherapy protocol used, or to general clinical parameters in mammary tumours and lymphomas of dogs (Dhaliwal et al., 2013; Gramer et al., 2013; Tomiyasu et al., 2014a; Zandvliet et al., 2015).

In a few studies, the desensitising effects of MDR1, BCRP, MRP1 activities have been directly confirmed in canine mammary tumour or lymphoma cells (Table 2). For instance, Pawlowski et al. (2013) were able to identify the drug specificity for different ABC transporters in a canine mammary tumour cell line. By siRNA-mediated gene silencing, they showed that: (1) vinblastine efflux was mediated by MDR1 and MRP1; (2) cisplatin efflux was mediated by MDR1, BCRP, MRP1; and (3) cyclophosphamide resistance was mediated by BCRP. In addition, doxorubicin and vincristine resistance in canine lymphoma cell lines was completely reversed by an MDR1 inhibitor (Zandvliet et al., 2014).