Regulation of the 24-Hydroxylase Gene Promoter by 1,25-Dihydroxyvitamin D3 and Chemotherapeutics Drugs.

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Thesis submitted for the degree of Doctor of Philosophy

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October 2005

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Joseph TanCheng Ta ***Dated***

Acknowledgement

Firstly, I would like to take this opportunity to express my sincere thanks to my principal supervisor, Associate Professor Charles Hii for his continuous supervision, support, encouragement and friendship which has made my PhD study an enjoyable experience.

I would also like to thank my co-supervisor Professor Antonio Ferrante for his guidance, patience, encouragement, friendship and support.

My special thanks to Dr Prem Dwivedi, with whom I worked closely, for his valuable supervision, great discussions and long lasting friendship. Also, special thanks to Professor Brian May, Professor Howard Morris and Dr Peter O'Loughlin for their guidance and for allowing me to use their laboratories to undertake some aspects of my study. Many thanks to Barbara Nutchey, Dr Yong Lee, Dr Sophia Gao and Dr Paul Anderson for their endless help, great discussion and friendship. A special thanks to all the PhD and honours students in the Department of Immunopathology for their great friendship and jokes that we shared. Also, many thanks to Judy Ferrante, Trish Harvey, Lily Zedda, Bernadette Miller, Jess Franks, Reneé Mackenzie and other members of the Department of Immunology, WCH, for their help and in making the laboratory a wonderful place to work in.

Last but not least, I would like to express my gratitude and thanks to God for his support, wisdom and strength, my wonderful family: my parents, my brothers and sisters for their continuous support and encouragement, to my dearest wife Ya-Hui and daughter Catrina, for their endless love, support, encouragement and for making my PhD years a meaningful and worthwhile experience.

Summary

Chemotherapy in childhood cancer patients is associated with reduced bone density that can result in osteoporotic fracture in survivors. A significant proportion of paediatric patients experience a reduction in plasma 25-hydroxyvitamin D3 [25(OH)D3] and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] levels during treatment, the basis of which is unknown. A balance between the bioactivation and degradation of 1,25(OH)2D3 is responsible for maintaining homoeostatic levels of 1,25(OH)2D3 at the correct set-point. Whereas the cytochrome P450 enzyme, CYP27B1 (25-hydroxyvitamin D3 1α-hydroxylase), catalyses the hydroxylation of the precursor 25(OH)D3 to generate 1,25(OH)2D3, catabolic inactivation and cleavage of 1,25(OH)2D3 is achieved by the mitochondrial cytochrome P450 enzyme, 25-hydroxyvitamin D3 24-hydroxylase (CYP24), which is highly expressed in bone and kidney cells. Since many of the signalling pathways which regulate the expression of CYP24 are also activated by chemotherapeutic drugs, we hypothesised that the drugs could cause the degradation of plasma 25(OH)D3 and 1,25(OH)2D3 by increasing CYP24 expression, the principal means of facilitating the bio-inactivation and degradation of plasma 25(OH)D3 and 1,25(OH)2D3. Using the kidney cell-lines, COS-1 and HEK293T cells, we now report that chemotherapeutic drugs, represented by daunorubicin hydrochloride (an anthracycline antibiotics), etoposide and vincristine sulphate (vinca alkaloids and related compounds) and cisplatin (an alkylating agent), were able to enhance CYP24 promoter activity in kidney cell lines transfected with a CYP24 promoter-luciferase construct, either by themselves or in the presence of 1,25(OH)2D3. Dose-response studies with

daunorubicin hydrochloride and etoposide, two of the strongest inducers of CYP24 promoter activation under our experimental conditions, demonstrate that these drugs acted in a concentration-dependent manner. In addition to stimulating promoter activity on their own, the drugs also amplified the induction of the CYP24 promoter by 1,25(OH)2D3. Synergistic increases were generally observed when the cells were treated simultaneously with 1,25(OH)2D3 and a drug. The two kidney cell lines generally responded in a similar manner when challenged with the drugs, either in the presence or absence of 1,25(OH)2D3. Interestingly, the hydroxylated derivative of daunorubicin hydrochloride, doxorubicin hydrochloride which is also a commonly used chemotherapeutic drug, had no effect of promoter activity. Further studies with daunorubicin hydrochloride demonstrated that the effects of the drug per se were not mediated by oxidative stress and the vitamin D receptor was not required for daunorubicin hydrochloride per se to stimulate CYP24 promoter activity. However, daunorubicin hydrochloride caused a modest increase in the expression of the vitamin D receptor and this could contribute to its synergistic activity with 1,25(OH)2D3. In the presence of etoposide, there was also a tendency for the kidney cells to express higher levels of the vitamin D receptor. A key role for the extracellular signal-regulated protein kinase (ERK)1, ERK2 and ERK5 mitogen-activated protein (MAP) kinases was demonstrated for the inductive action of daunorubicin hydrochloride and etoposide, with CYP24 promoter-specific transcription factors located in the first –298bp being likely targets of the ERK activity. Studies with a dominant negative mutant of MKK4, one of the two immediate upstream activators of the c-jun N-terminal kinase isoforms, demonstrated that this MAP kinase also played a crucial role in inductive actions of the

drugs. Consistent with their use in anti-cancer therapy, all of the above drugs killed the human promyelocytic HL60 leukaemic cells at very low concentrations but had no effect on the viability of kidney or liver cells, either at concentrations used in our experiments or at higher levels. Our data provide novel biochemical evidence that some of the commonly used chemotherapeutic drugs could cause an increase in the transcriptional activation of the promoter, most likely via the MAP kinases activating the transcription factors which bind to the CYP24 promoter. Such an effect could contribute to the reduction in plasma 25(OH)D3 and 1,25(OH)2D3 in some of the patients undergoing chemotherapy.

Abreviations

1,25(OH)2D3/ 1,25D	1,25-dihydroxyvitamin D3
24,25(OH)2D2	24,25-dihydroxyvitamin D3
25(OH)D3	25-hydroxy vitamin D3
AP1	activator protein 1
BDNF	brain-derived neurotrophic factor
CaT1	calcium transport protein
COS-1	African monkey kidney fibroblast cells
Cx43	connexin 43; a gap junction protein
CYP24	24-hydroxylase enzyme
CYP27b1	1α-hydroxylase enzyme
DN	dominant negative
EBS	Ets-1 binding site
ECaC2	epithelial calcium channel protein
EGF	epithelial growth factor
ERK	extracellular signal-regulated kinase
ERK5	Big MAP kinase 1
EtOH	ethanol
Ets	E26
FGF-2	fibroblast growth factor-2
HEK293T	transform human embryonic kidney cells
HL60	human promyelocytic leukaemic
HPV	human papillomaviruses

IL	interleukin
JNK	c-Jun NH_2-terminal kinases
LY294002	PI3K inhibitor
MAPK	mitogen-activated protein kinase
MEF2	myocyte enhancer factor 2
NCX1	Na^+/Ca^{2+} exchanger pump
NF-Y	transcription factor nuclear factor Y
NGF	nerve growth factor
OPG	osteoprotegerin gene
PDK	phosphoinositide-dependent kinase
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PKC	protein Kinase C
PMA	phorbol 12-myristate 13-acetate
PMCA	plasma membrane calcium ATPase
PTH	parathyroid hormone
RANKL	NFkappa-B ligand
Ras	small GTP-binding and hydrolyzing proteins
ROS	reactive oxygen species
RXR	retinoic X receptor
Sap1	Ets-domain transcription factor
SGK	serum- and glucocorticoid-inducible kinase
Sp1	specific protein 1

TGF-β	transforming growth factor-beta
Th	T helper
Tpl	tumor progression locus
TRPV	transient receptor potential vanilloid
VDR	vitamin D receptor
VDRE	vitamin D responsive element
VEGF	vascular endothelial growth factor
VP-16	etoposide
VSE	vitamin D stimulatory element

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