The Effects of Tyrosine Kinase Inhibition on Bone Remodelling

Kate Vandyke

Myeloma Research Laboratory
Bone and Cancer Laboratories
Department of Haematology
Institute of Medical and Veterinary Science
&
Department of Medicine
Faculty of Health Sciences
The University of Adelaide

A thesis submitted to the University of Adelaide
for the degree of Doctor of Philosophy
July 2010

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Abstract

Imatinib is a rationally-designed tyrosine kinase inhibitor that is a highly-successful treatment for chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours. In a retrospective study, it was previously shown that imatinib therapy is associated with an increase in trabecular bone volume. In this current study, a prospective analysis of bone indices in imatinib-treated CML patients was carried out to determine the mechanism responsible for this altered bone remodelling. Imatinib therapy resulted in an increase in trabecular bone volume and trabecular thickness in iliac crest trephines, relative to prior to treatment. This was associated with a significant decrease in osteoclast numbers, assessed histologically, and in serum levels of a marker of osteoclast activity. Osteoblast numbers were not altered by up to 12 months of treatment. These data suggest that imatinib dysregulates bone remodelling by inhibiting osteoclast activity.

It was next examined whether a second-generation tyrosine kinase inhibitor, dasatinib, which is successfully used to treat CML in imatinib-resistant patients, could similarly alter bone remodelling. Dasatinib treatment significantly increased trabecular bone volume and trabecular thickness in a rat model of normal bone remodelling. This was primarily attributable to inhibition of osteoclast activity, at least in part through inhibition of Fms. These studies show for the first time that dasatinib treatment is associated with a decrease in osteoclast formation and activity *in vitro* and *in vivo*, suggesting that decreased bone resorption is a likely side-effect of dasatinib therapy. Additionally, these studies highlight the possibility that both imatinib and dasatinib may represent potential treatments for diseases characterised by aberrant bone loss.

While imatinib is well-tolerated in children, emerging data suggest that long-term therapy may result in decelerated growth in juvenile CML patients. To date, there are no reports suggesting a mechanism for altered growth in imatinib-treated paediatric patients. In a normal mature rat model, we found that daily treatment with imatinib or dasatinib significantly decreased growth plate thickness at the proximal tibia, with a complete fusion of the growth plate by 12 weeks of treatment in imatinib-treated animals. Furthermore, chondrocyte proliferation and activity were significantly decreased by imatinib and dasatinib treatment *in vitro*, through a mechanism that may involve inhibition of PDGFRβ. The dramatic effect of imatinib and dasatinib on growth plate morphology in rats suggests that growth plate closure should be investigated as a potential mechanism for inhibited growth in pre-pubescent patients receiving imatinib.

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. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed

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Kate Vandyke

Acknowledgements

First I would like to thank my wonderful supervisors A/Prof. Andrew Zannettino, Dr Stephen Fitter and Dr Andrea Dewar. Thanks to Andrew Zannettino for his unending optimism, praise and support throughout this project and for generally being the best supervisor I could hope for. I am very grateful to Steve Fitter for always being available to bounce ideas off and to answer my many inane questions. I very much appreciate him taking the time to go through the nitty gritty details of all my writing, picking up on the smallest possible errors. Any mistakes that have made it into this thesis are entirely my own! Thanks also must go to Dr Andrea Dewar for always being there to offer guidance and support throughout my PhD, even at the times when I'm sure she would rather have forgotten all about science!

I am forever grateful to all my colleagues in the Bone and Cancer Laboratories for being such wonderful bunch of people and for making work a happy, fun and, sometimes, crazy place to be. Special thanks need to go to a few of you: Dr Peter "don't you know who I am?" Diamond for all his help with animal experiments, particularly for stepping in and helping scan my rats on those days when I'd been going non-stop all day and really needed to break for noodles; to Jenny Drew for conducting beautiful histology on the patient bone samples; to Dr Sally "the font of all knowledge" Martin for always being there with the answer to any possible question (she is truly all-knowing); and to Danijela "Danni-Julia" Menicanin for all her help with histology and for love, hugs and good will going through the crazy write-up time together with me - it has helped tremendously to have someone to share this process with. I also must give a big thanks to Dr Tony Cambareri for all his help with printing my thesis; his expertise helped everything go a whole lot more smoothly. Thank you also to the rest of the lab members, past and present: Dr Agnes Arthur, Amanda Davis, Catherine Gan, Dr Jim Cakouros, Jimin Xiong, Kate Pilkington, Dr Kim Hynes, Krzysztof Mrozik, Locky Cooper, Mary Matthews, Dr Naohisa Wada, Penny Kostakis, Dr Peter Psaltis, Romana Panagopoulos, Sandra Isenmann, Dr Sharon Williams, Sharon Paton and A/Prof. Stan Gronthos; I truly could not have asked for a better group to work with.

Among the many other people I am indebted to for help with this project, I must single out a few individuals: Peter McNeil and Rebecca Sawyer for teaching me the ins and outs of methacrylate embedding; Peter Self at Adelaide Microscopy for always being available

with help and advice when µ-CT wasn’t going as it should; Chris Schultz from the Royal Adelaide Hospital for carrying out all the DXA scans of my rats and patients; Natalie Sims at St Vincent's Institute for passing on her not inconsiderable knowledge of bone histomorphometry and for letting me hole myself up in her microscope room on several occasions to analyse slides for days on end. Cheers also to all the staff at Veterinary Services, IMVS, especially Brian Lewis, Brigitt Hines, Kelly Wicks, Briony Gliddon, Dr Tim Kuchel and of course to Behzad Baradaran who did such a great job gavaging my rats. I’d also like to thank the staff at Haematology Clinical Trials, in particular Lisa Carne and Christine Hoare, who did a tremendous job organising the TIDEL II study. Particular thanks must also go to all the patients who donated their time and body parts to my study: you are all amazing.

Thanks to my parents Tina and John and Mel for all your love, support and generosity throughout the past 8 years of university. Without your help I never could have made it this far and I am eternally grateful for it. To my sister Jen for letting me stay on her couch in Melbourne, for being available for hours of telephone calls when I needed to unwind and for understanding when I didn’t have the time to call. I also need to give a heartfelt thanks to my substitute parents in Adelaide, Julie and Tony Atkins, for doing such a great job caring for and, particularly, feeding James, Joe and I, when I haven’t had the time to do it! I am so very lucky to have such wonderful in-laws.

The biggest thank you must go to my incomparable partner James for his inexhaustible patience, encouragement and support (among rather a lot else).

Thank you

Abbreviations

ABC	adenosine triphosphate-binding cassette transporter
Abl	Abelson kinase
AHSA1	activator of heat shock 90 kDa protein ATPase homologue 1
ALL	acute lymphoblastic leukaemia
α-MEM	α-modified Eagle's medium
ANOVA	analysis of variance
APS	ammonium persulphate
ARG	Abl-related gene
ATCC	American Type Culture Collection
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
bALP	bone-specific alkaline phosphatase
Bcr	breakpoint cluster region
BCRP	breast cancer resistance protein
BFR	bone formation rate
BID	*bis in die*
BM	bone marrow
BMC	bone mineral content
BMD	bone mineral density
BMP	bone morphogenetic protein
BMU	basic multicellular unit
B.Pm	bone perimeter
BS	bone surface
BSA	bovine serum albumin
BV	bone volume
CA	carbonic anhydrase
c-α-MEM	complete α-modified Eagle's medium
CBF-β	core-binding factor-β
Cbl	Cas-Br-M ecotropic retroviral transforming sequence
c-DMEM	complete Dulbecco's modified Eagle's medium
C/EBP	CCAAT/enhancer binding protein
CML	chronic myeloid leukaemia

CPC	cetyl pyridinyl chloride
CSA	chondroitin sulphate A
CSF1	colony stimulating factor 1
CSF1R	colony stimulating factor 1 receptor
Ct.BV	cortical bone volume
Ct.Th	cortical thickness
CTX-1	C-terminal collagen crosslinks
DDR	collagen-induced discoiddin domain receptor
Dkk	Dickkopff family member
DMEM	Dulbecco's modified Eagle's medium
DMSO	dimethyl sulphoxide
DTT	dithiothreitol
DXA	dual energy X-ray absorptiometry
ECF	enhanced chemifluorescence
EDTA	ethylenediaminetetraacetic acid
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbent assay
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FCS	foetal calf serum
FGF	fibroblast growth factor
Fms	McDonough feline sarcoma viral oncogene homologue
FOS	Finkel-Biskis-Jinkins murine osteosarcoma viral oncogene homologue
FOXO	forkhead box class O
GAG	glycosaminoglycan
GFR	glomerular filtration rate
GH	growth hormone
GIST	gastrointestinal stromal tumours
Grb2	growth factor receptor-bound protein 2
GSK-3β	glycogen synthase kinase-3β
HBSS	Hanks' buffered saline solution
HCl	hydrochloric acid
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid

HLA	human leukocyte antigen
HPLC	high-performance liquid chromatography
HSP90	heat shock protein 90 kDa
IC_{50}	inhibitory concentration, 50%
Ig	immunoglobulin
IGF	insulin-like growth factor
Ihh	Indian hedgehog
IKK	IκB kinase
IL	interleukin
IP	intraperitoneally
IRIS	International Randomized Study of Interferon and STI571
JNK	Jun N-terminal kinase
Kit	stem cell factor receptor
Lef	lymphoid enhancer-binding factor
LRP	low density lipoprotein receptor-related protein
MAP2K	mitogen-activated protein kinase kinase
MAR	mineral apposition rate
mBM	mouse bone marrow monocyte
M-CSF	macrophage colony stimulating factor
µ-CT	micro-computed tomography
MDR1	multidrug resistance-1
MEK	extracellular signal-regulated kinase kinase
min.BV	mineralised bone volume
Mitf	microphthalmia-associated transcription factor
mRNA	messenger ribonucleic acid
MS	mineralised surface
NBF	neutral buffered formalin
NFAT	nuclear factor for activated T cells
NF-κB	nuclear factor of κ light polypeptide gene enhancer in B-cells
N.Ob	number of osteoblasts
N.Oc	number of osteoclasts
NQO2	nicotinamide adenine dinucleotide phosphate-oxidase:quinone oxidoreductase 2
NTX	N-telopeptide of collagen crosslinks

Ob.S	osteoblast surface
Oc.S	osteoclast surface
OCT-1	organic cation transporter-1
OPG	osteoprotegerin
OS	osteoid surface
O.Th	osteoid thickness
OV	osteoid volume
P1NP	pro-collagen type I amino-terminal propeptide
PBS	phosphate buffered saline
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PEG	polyethylene glycol
PI3K	phosphoinositide-3-kinase
PMSF	phenylmethylsulphonyl fluoride
pQCT	peripheral quantitative computed tomography
PTH	parathyroid hormone
PTHrP	parathyroid hormone-related protein
PVDF	polyvinylidene difluoride
Rac	Ras-related C3 botulinum toxin substrate 1
RANK	receptor activator of nuclear factor-κB
RANKL	receptor activator of nuclear factor-κB ligand
rBM	rat bone marrow monocyte
rBMSC	rat bone marrow stromal cell
rh	recombinant human
RO	reverse osmosis
Runx2	runt-related transcription factor 2
RXR	retinoic acid X receptor
SCF	stem cell factor
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
sf-α-MEM	serum-free α-modified Eagle’s medium
sf-DMEM	serum-free Dulbecco’s modified Eagle’s medium

sFRP	secreted Frizzled-related protein
Smad	mothers against decapentaplegic homologues
SMI	structure model index
Sox	sex determining region Y box
SPI1	spleen focus forming virus proviral integration oncogene
sRANKL	soluble receptor activator of nuclear factor-κB ligand
Src	sarcoma viral oncogene homologue
Tb.BMD	trabecular BMD
Tb.N	trabecular number
Tb.Pf	trabecular pattern factor
TBS	Tris-buffered saline
Tb.Sp	trabecular spacing
TBS-Tween	Tris-buffered saline with 1% Tween 20
Tb.Th	trabecular thickness
Tcf	T-cell-specific transcription factor
TEMED	N,N,N,N-tetramethylethylenediamine
TGF-β	transforming growth factor-β
TIDEL	Therapeutic Intensification in *de Novo* Leukaemia
TmP	maximum tubular resorption of phosphate
TNF	tumour necrosis factor
TRAF6	tumour necrosis factor receptor-associated factor 6
TRAP	tartrate-resistant acid phosphatase 5
TV	total volume
UV	ultraviolet
VDR	vitamin D receptor
Wif	Wnt inhibitory factor
Wnt	wingless-type mouse mammary tumour virus integration type family member
WST-1	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate

Publications

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