
Experimental Investigations into Sarcomas

Manfred Georg Krukemeyer

Experimental Investigations into Sarcomas

Therapy Using Ferromagnetically
Induced Cytostatics



Springer

Manfred Georg Krukemeyer
Osnabrück, Germany

English Translation of the German edition published by Springer 2018, „Experimentelle Untersuchungen bei Sarkomen – Therapie mittels ferromagnetisch induzierter Zytostatika“, Springer, 2018, ISBN 978-3-658-20254-5

Original German edition published by Springer, Wiesbaden, 2018

ISBN 978-3-658-20590-4 ISBN 978-3-658-20591-1 (eBook)
<https://doi.org/10.1007/978-3-658-20591-1>

Library of Congress Control Number: 2017963267

© Springer Fachmedien Wiesbaden GmbH 2018

This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Springer imprint is published by Springer Nature

The registered company is Springer Fachmedien Wiesbaden GmbH

The registered company address is: Abraham-Lincoln-Str. 46, 65189 Wiesbaden, Germany

Table of Contents

List of Figures.....	VII
List of Tables.....	IX
List of Abbreviations.....	XI
1 Introduction	1
2 General	5
2.1 Sarcomas and their Pathology	5
2.1.1 Soft Tissue Sarcomas.....	5
2.1.2 Malignant Bone Tumours	9
2.2 Epidemiology of Sarcomas.....	15
2.3 Tumour Treatments	17
2.3.1 General.....	17
2.3.2 Chemotherapy.....	18
2.4 Magnetic Drug Targeting	19
2.5 Iron Metabolism	21
3 Material and Method	23
3.1 Drug Administration System.....	23
3.1.1 Drug	23
3.1.2 Drug Delivery	24
3.1.3 Magnets	28
3.2 Animal Studies	31
3.2.1 General.....	31
3.2.2 Study Animals	32
3.2.3 Study Design.....	32
3.2.4 Tumour Model.....	36
3.2.5 Narcosis Method.....	37
3.2.6 Surgical Intervention	37
3.2.7 Histology	39
3.2.8 Histopathological Evaluation.....	40
3.2.9 Laboratory Analysis of Iron and Mitoxantrone	40

3.3 Study on Patients	40
3.3.1 Individual Compassionate Use	43
3.3.2 Evidence of Volume Reduction by Magnetic Resonance Imaging	43
3.3.3 Pathohistology and Laboratory Analysis Measurement Methods	44
3.4 Statistics.....	45
4 Results.....	47
4.1 Animal Studies	47
4.1.1 Biodistribution of Mitoxantrone	47
4.1.2 Results of the Dose/Effect Study Groups	50
4.1.3 Iron distribution in the rat	54
4.1.4 Macroscopic Images of the Rat Studies.....	56
4.1.5. Histopathology Results.....	57
4.2 Tumour Treatment in Humans.....	62
4.2.1 Tumour Treatment in Patient L.K.....	62
4.2.2 Tumour Treatment in Patient J.W.....	73
5 Discussion	85
5.1 Biodistribution of Nanoparticles.....	85
5.2 Measurement Methods for Tumour Size and Uptake of Fe ₃ O ₄ in the Tumour.....	86
5.3 Concentration of Iron and Cytostatics in Tumour Treatment.....	87
5.4 Cytostatics and Their Side Effects.....	90
5.5 Tumour Reduction and Tumour Recurrence	92
5.6 Conclusions	94
6 Summary	97
Literature	99
Anhang	115

List of Figures

Figure 1:	Microphotograph of HE standard stain	9
Figure 2:	Macroscopic aspect of an osteosarcoma	10
Figure 3:	Cortical/periosteal tumour location.....	11
Figure 4:	Bone tumours	12
Figure 5:	Macroscopic aspect of an osteosarcoma	15
Figure 6:	The 20 most frequent types of cancer deaths in 2003	16
Figure 7:	Coupling of mitoxantrone with nanoparticles.....	25
Figure 8:	Magnetic field and poles.....	29
Figure 9:	The magnetic field.	29
Figure 10:	Magnet and magnetic field in the tumour.	30
Figure 11:	Extravasation of the nanoparticles in the tumour tissue.....	31
Figure 12:	Detailed view of the magnet over the tumour in the rat.....	32
Figure 13:	Wag/Rij rats under a magnetic field – study design.....	37
Figure 14:	Wag/Rij rats under a magnetic field – study design.....	38
Figure 15:	Wag/Rij rats under a magnetic field – study design.....	38
Figure 16:	Arrangement of the magnets over the anaesthetised animals..	39
Figure 17:	Magnet with holder.	41
Figure 18:	Detailed view of the magnet.	42
Figure 19:	Mitoxantrone – biodistribution in the blood (plasma).....	48
Figure 20:	Mitoxantrone – biodistribution in the tumour.....	49
Figure 21:	Development of the tumour volume.	50
Figure 22:	Iron content in the blood (plasma) of the rat.....	54
Figure 23:	Exophytically growing tumour – rhabdomyosarcoma in the rat after removal of the skin.	56

Figure 24:	Exophytically growing tumour – rhabdomyosarcoma after tissue resection.....	57
Figure 25:	Skin/subcutaneous tumour.	58
Figure 26:	Regular morphology of the liver parenchyma.....	58
Figure 27:	Regular hepatic parenchyma	59
Figure 28:	Liver: Regular morphology of the hepatic parenchyma.....	59
Figure 29:	Histopathology of the tumour of the dose/effect study/rat tumour	60
Figure 30:	Histopathology of the tumour of the dose/effect study/rat tumour	60
Figure 31:	Histopathology of the tumour of the dose/effect study/rat tumour.	61
Figure 32:	Histopathology of the tumour of experiment X	61
Figure 33:	Spleen: Regular architecture of the hepatic parenchyma in the red pulp and perifollicular	62
Figure 34:	30.05.2008 series 4 image 18 – pre.....	71
Figure 35:	04.07.2008 series 6 image 18 – post.	72
Figure 36:	30.05.2008 series 5 image 25 – pre.....	72
Figure 37:	04.07.2008 series 7 image 23 – post.	73
Figure 38:	Coronary MRI layers of the thorax, T2-emphasised.....	82
Figure 39:	Coronary MRI layers of the thorax, T2-emphasised	82

List of Tables

Table 1:	Vienna tumour stages.....	18
Table 2:	Advantages of targeted drug administration	20
Table 3:	Physiology of iron metabolism.	22
Table 4:	Sensitivity of the sarcomas to radiation and chemotherapy. 24	
Table 5:	Laboratory data	26
Table 6:	Specification of MagnaDrug.....	27
Table 7:	Study groups in the biodistribution.....	33
Table 8:	Target values and parameters investigated.	33
Table 9:	Groups of the dose/effect trial.....	33
Table 10:	Target values and parameters investigated.	34
Table 11:	Iron distribution study group.	35
Table 12:	Target values and parameters investigated.	36
Table 13:	Descriptive statistics methods.....	45
Table 14:	Mitoxantrone – biodistribution in the blood (plasma)	47
Table 15:	p values for comparison of the mitoxantrone concentration in the blood (plasma) between groups I–VI.....	48
Table 16:	Mitoxantrone – biodistribution in the tumour.....	48
Table 17:	p values for comparison of the mitoxantrone concentration in the tumour between groups I–VI.....	50
Table 18:	Tumour volume day 1.....	51
Table 19:	Tumour volume day 8.....	51
Table 20:	Dose/effect study – group comparison of the p values on day 8.....	52
Table 21:	Development of the tumour volume.	52

Table 22:	Dose/effect study – Comparison of day 8 to day 1	52
Table 23:	Dose/effect study – comparison of the volumes at the end of the study.....	53
Table 24:	Comparison of the tumour volumes at the end of day 8 minus starting volume of day 1 – comparison of the medians between the groups	53
Table 25:	Iron content in the blood of the rat.....	55
Table 26:	Iron content blood.....	55
Table 27:	Iron content in the blood of the rat.....	56
Table 28:	Complications.	65
Table 29:	Blood sampling on the 4th day.	68
Table 30:	Patient data.....	68
Table 31:	Iron oxide particles and ferritin on day -2, 0, 1, 2, 3 and 4 and after 2 weeks.....	69
Table 32:	Blood values.....	69
Table 33:	Complications.	77
Table 34:	Blood sampling on the 4th day.	79
Table 35:	Patient data.....	80
Table 36:	Iron oxide particles and ferritin on day -2, 0, 1, 2, 3 and 4 and after 2 weeks.....	80
Table 37:	Blood values.....	80
Table 38:	Overview of the most important osteosarcoma entities	115
Table 39:	Incidence peak of benign and malignant tumours and tumorous lesions	117
Table 40:	Calculation of Body Surface Area (BSA).....	118

List of Abbreviations

Sec.	Section
ADM	Adriamycin
Ab	Antibody
AMG	Medicinal Products Law
B	Flux density
BBodSchG	Federal Soil Protection Act
BGN	Betaglycan
BSN	Bone sialoprotein
CCT	Cranial computerised tomogram
CD	Cluster of differentiation
COSS study	Cooperative Osteosarcoma Study Group
CT	Computerised tomogram
DCN	Decorin
DIN	German Industry Standard
DNA	Deoxyribonucleic acid
EGF	Endothelial growth factor
G ₂ phase	Premitotic phase, postsynthesis phase
H	Field strength
HE	Haematoxylin/eosin
HER2	Human epidermal growth factor
IFN- γ	γ -Interferon
IgG	Immunoglobulin
ILP	Isolated limb perfusion
In	Indium
BW	Body weight
KI 67	Proliferation marker 67
BSA	Body surface area
MDR-1 gene	Multiple drug resistance gene
MFH	Malignant fibrohistiocytic tumour
M phase	Mitosis phase

MR layers	Magnetic resonance layers
MRI	Magnetic resonance imaging
MTX	Methotrexate
n	Number
NOS	not otherwise specified
Nx	Compound of nitrogen with a halogen
OC	Osteocalcin
OPN	Osteopontin
T	Trial group
PVC	Polyvinyl chloride
R ₀ phase	Complete removal of the tumour
R ₁ H	Rhabdomyosarcoma
rb gene	Retinoblastoma gene
Re	Rhenium
RES	Reticuloendothelial system
RNA	Ribonucleic acid
S100	Calcium-binding proteins
S3 Laboratory	Laboratory with safety level 3
Sm	Samarium
Sr	Strontium
Std Dev	Standard deviation
SV-40 virus	Simian virus 40
T [min]	Time in minutes
T ₆₁	Embutramide
Tc (^{99m})	Technetium (^{99m})
TNF α	Tumour necrosis factor α
TNM	Tumour node metastasis
C	Comparison group
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation
CNS	Central nervous system
μm	10^{-6} m
nm	10^{-9} m
γ_0	Field constant