



Therapeutic Potential of Human Umbilical Cord Blood Derived Mesenchymal Stem Cells in Murine Model of Hepatic Schistosomiasis

Thesis

Submitted to Faculty of Medicine, Ain Shams University for Partial
Fulfillment of M.D Degree in Medical Science (Parasitology)

By

Shaimaa Essam Al-din Mohammed

M.B, B.Ch, M.Sc

Assistant Lecturer of Medical Parasitology
Faculty of Medicine, Ain shams university

Under Supervision of

Professor/Mohammad Abd-El Rehiem Bebars

Professor of Medical Parasitology
Faculty of Medicine , Ain Shams University

Professor/ Hanan Hussein Kamel

Professor of Medical Parasitology
Faculty of Medicine , Ain Shams University

Ass. Prof. / Heba Abdel Kader Aminou

Assistant Professor of Medical Parasitology
Faculty of Medicine , Ain Shams University

Ass. Prof. / Lobna Sadek Shash

Assistant Professor of Pathology
Faculty of Medicine , Ain Shams University

Dr. / Ayman Mohammad Abd El Azim Al-Ashkar

Lecturer of Medical Parasitology
Faculty of Medicine, Ain Shams University

Medical parasitology Department

**Faculty of Medicine
Ain Shams University**

2019

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

لسبب انك لا تعلم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢



ACKNOWLEDGEMENT

First of all, thanks to **Allah** whose magnificent help was the main factor in completing this work.

*Words cannot express my deepest gratitude to **PROF. DR. MOHAMMAD ABD-EL REHIEM BEBARS**, Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University, who helped me throughout this work by training, precious instructions, valuable advices and scientific knowledge to accomplish such work.*

*It has been a great honor for me to work under her generous supervision. Great words really needed to express my gratitude, sincere appreciation and respect to **PROF. DR. HANAN HUSSEIN KAMEL**, Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University, for her great help, continuous support, and sincere advice during this work.*

*I would also like to thank **ASS. PROF. DR. HEBA ABDEL KADER AMINOU**, Assistant Professor of Medical Parasitology, Faculty of Medicine, Ain Shams University for her continuous encouragement, guidance and support.*

*I would also like to thank **ASS. PROF. DR. LOBNA SADEK SHASH**, Assistant Professor of Pathology, Faculty of Medicine, Ain Shams University for her continuous guidance and support she gave me throughout the whole work.*

*I would also like to thank **DR. AYMAN MOHAMMAD ABD EL AZIM AL-ASHKAR**, Lecturer of Parasitology, Faculty of Medicine, Ain Shams University, Medical parasitology Department, for his help and valuable advice.*

Finally, my deepest thanks to all my family and colleagues who helped me in the production of this work.

Contents

Subjects	Page
List of Abbreviations.....	II
List of Figures.....	V
List of Graphs.....	XI
List of Tables.....	IX
Abstract.....	XII
• Introduction	1
• Review of Literature	4
♦ <i>Schistosoma mansoni</i>	4
♦ <i>Schistosomiasis mansoni</i>	9
♦ Stem cells.....	37
• Aim of the Work	77
• Material and Methods	78
• Results	103
• Discussion	138
• Summary and Conclusion	156
• Recommendations	161
• References	162
• Arabic Summary	

List of Abbreviations

ALT	Alanine aminotransferase
AMI	Acute myocardial Infarction
ART	Artemether
AST	Aspartate aminotransferase
BFU-E	Burst forming unit erythroid
BMT	Bone marrow transplantation
Cd	Cercarial dermatitis
CD	Cluster of differentiation
CF	Combined fibrosis
CFU-GEMM	Colony forming unit granulocyte erythrocyte monocyte megakaryocytic
CFU-GM	Colony forming unit granulocyte monocyte
CMI	Cell mediated immunity
DCM	Dilated cardiomyopathy
DMEM	Dulbecco's Modified Eagles Medium
DPBS	Diluted phosphate buffer saline
ECM	Extracellular matrix
EDTA	Ethylene Diamine Tetra Acetate
EGF	Epidermal growth factor
ESC	Embryonic stem cells
FABP	Fatty acid binding protein
FBS	Fetal bovine serum

List of Abbreviations

FITC	Fluorecein isocyanate
GFAP	Glial Fibrillary Acidic Protein
GPx	Glutathione peroxidase
GST	Glutathione transferase
GVHD	Graft-Versus-Host Disease
H&E	Haematoxylin and eosin
HBSS	Hank`s Balanced Salt Solution
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HLA	Human Leukocyte antigens
HME	Histone modifying enzymes
HSCs	Hematopoietic stem cells
HSCs	Hepatic stellate cells
HUC-MSCs	Human umbilical cord blood mesenchymal stem cells
IBD	Inflammatory bowel disease
IFN-beta	Interferon beta
IFN-γ	Interferon gamma
IgG2	Immunoglobulin G2
IL	Interleukin
IMDM	Iscove`s modified Dulbecco`s medium
IPSCs	Induced pluripotent stem cells
IRB	Institutional Review Board

List of Abbreviations

KS	Katayama syndrome
MD-SCs	Muscle-derived stem cells
MELD	Model for End-Stage Liver Disease
MNCs	mononuclear cells
MPCs	Multipotential mesenchymal progenitor cells
MS	Multiple sclerosis
MSCs	Mesenchymal stem cells
MT	Masson Trichome
Na-APR-1	<i>Necator americanus</i> aspartic protease
NIH	National institute of health
NK cells	Natural Killer cells
Non-ESC	Non-embryonic stem cells
PBS	Phosphate buffered saline
P-C	Porto-central
PE	Phycoerythrin
PGE1	Prostaglandin E1
PGF	Perigranulomatous fibrosis
P-P	Porto-portal
PT	Portal tract
PZQ	Praziquantel
RV	Right ventricle
<i>S. mansoni</i>	<i>Schistosoma mansoni</i>

List of Abbreviations

SEA	Soluble egg antigen
Sm-TSP-2	<i>Schistosoma mansoni</i> tetraspanin
SOD	Superoxide dismutase
SWAP	<i>Schistosoma</i> worm adult protein
TBRI	Theodor Bilharz Research Institute
TF	Totally fibrosed
TNF-α	Tumor Necrosis Factor Alpha
Tregs	Regulatory T-cells
UCB	Umbilical cord blood
WHO	World health organization
α-SMA	Alpha Smooth Muscle Actin

List of Figures

<u>Figure</u>	<u>Title</u>	<u>Page</u>
<u>1</u>	<i>Schistosoma mansoni</i> adult male and female.	5
<u>2</u>	<i>Schistosomes</i> life cycle.	8
<u>3</u>	(A) Acute liver granuloma around <i>S. mansoni</i> egg in liver of an experimentally infected mouse containing many cells, mainly eosinophils, lymphocytes and some macrophages; large red cells are intact murine hepatocytes. (B) Chronic liver granuloma around the remains of a <i>S. mansoni</i> egg in a mouse liver, with dominant (blue colored) fibrous tissue	14
<u>4</u>	The major components of the schistosome induced granulomas and the cytokines that drive this response.	27
<u>5</u>	Embryonic stem cells & Stem cell therapy.	40
<u>6</u>	Sample collected and processed immediately after deliveries.	81
<u>7</u>	UCB samples were collected from the umbilical vein using heparinized syringes.	82
<u>8</u>	The diluted umbilical cord blood layered over an equal volume of Ficoll-HyPaque.	82

List of Figures

<u>Figure</u>	<u>Title</u>	<u>Page</u>
<u>9</u>	After centrifugation Mononuclear cells (MNCs) were recovered from the gradient interface.	83
<u>10</u>	Laminar flow apparatus.	86
<u>11</u>	Flasks containing the cultured stem cells.	88
<u>12</u>	Morphology of cells after 6 hours resuspended in medium.	105
<u>13</u>	Primary culture of CB-MSCs on day two of isolation showing adherent cells.	105
<u>14</u>	Fibroblast-like shape of mesenchymal stem at day 4 of isolation	106
<u>15</u>	On days 7 after initial plating, the cells took on the appearance of long spindle-shaped fibroblastic cells, began to form colonies.	106
<u>16</u>	Cells grew in colonies, reaching confluence after 14 days.	107
<u>17</u>	Immunophenotypes of CB- MSC.	108
<u>18</u>	Hepatic tissue section, group B3 with 9 predominantly cellular granulomas of average size score 2 and granulomas fusion.	109
<u>19</u>	Hepatic tissue section, group A1 with 6 granulomata, starting granulomatous evolution (arrow head) average size score 2.	110
<u>20</u>	Hepatic tissue section, group B3 showing 2 granulomata with average size score 3.	110

List of Figures

<u>Figure</u>	<u>Title</u>	<u>Page</u>
<u>21</u>	Hepatic tissue section, group A3 showing only two predominantly fibrotic (left is burnt out) granulomas with average size score 1, bile ducts show no hyperplasia (arrow head).	111
<u>22</u>	Hepatic section in group B3 with marked hyperplastic intra-hepatic billiary radicals (arrow heads).	117
<u>23</u>	Hepatic section in A1 group with marked hyperplastic intra-hepatic billiary radicals (arrows), granuloma size score 2, totally fibrotic.	118
<u>24</u>	Hepatic tissue section group B3 showing marked peri-granulomatous fibrosis with granulomas fusion.	122
<u>25</u>	Hepatic section group A3 with mild peri-granulomatous fibrosis along peripheral granuloma rim.	123
<u>26</u>	Hepatic section group A3 with fibrotic involuted granulomas and minimal peri-granulomatous fibrosis along peripheral granuloma rim at arrow heads.	123
<u>27</u>	Hepatic section group B2 with fibrocellular granulomas and extensive peri-granulomatous fibrosis and evident P-P bridging.	124
<u>28</u>	Hepatic section group B2 with fibrocellular granulomas and peri-granulomatous fibrosis with granulomatous fusion (arrow).	124

List of Figures

<u>Figure</u>	<u>Title</u>	<u>Page</u>
<u>29</u>	Hepatic tissue section group B3 showing marked fibrosis with granuloma fusion and p-p bridging.	125
<u>30</u>	Hepatic section in group B3 with severe fibrosis and p-p bridge highlighted by bluish stain (arrow).	125
<u>31</u>	Hepatic section of group B1 with moderate peri-granulomatous and sinusoidal SMA staining (note associated biliary hyperplasia: arrow head).	130
<u>32</u>	Hepatic section group B2 with strong diffuse SMA staining.	130
<u>33</u>	Hepatic section group B3 with diffuse strong SMA staining.	131
<u>34</u>	Hepatic section group A1 with moderate granulomatous and sinusoidal SMA staining.	131
<u>35</u>	Hepatic section group A2 with weak or moderate sinusoidal SMA staining.	132
<u>36</u>	Hepatic section groupA3 with very weak SMA staining.	132

List of Graphs

<u>Graph</u>	<u>Title</u>	<u>Page</u>
<u>1</u>	Comparison between group A1 and group B1 regarding granuloma size.	112
<u>2</u>	Comparison between group A2 and group B2 regarding granuloma size.	112
<u>3</u>	Comparison between group A3 and group B3 regarding granuloma size.	113
<u>4</u>	Comparison between group A1 and group A3 regarding granuloma size.	114
<u>5</u>	Comparison between control and test groups regarding mean granuloma number per 100 magnification power field.	116
<u>6</u>	Comparison between group A1 and group B1 regarding hyperplastic billiary changes.	119
<u>7</u>	Comparison between group A2 and group B2 regarding hyperplastic billiary changes.	119
<u>8</u>	Comparison between group A3 and group B3 regarding hyperplastic billiary changes.	120
<u>9</u>	Comparison between group A1 and group A3 regarding hyperplastic changes of intra-hepatic billiary radicals.	121

List of Graphs

<u>Graph</u>	<u>Title</u>	<u>Page</u>
<u>10</u>	Comparison between group A1 and group B1 regarding peri-granulomatous fibrosis (H&E-MT).	126
<u>11</u>	Comparison between group A2 and group B2 regarding peri-granulomatous fibrosis (H&E-MT).	127
<u>12</u>	Comparison between group A3 and group B3 regarding peri-granulomatous fibrosis (H&E-MT).	127
<u>13</u>	Comparison between group A1 and group A3 regarding peri-granulomatous fibrosis (H&E-MT).	129
<u>14</u>	Comparison between group A1 and group B1 regarding SMA (in granuloma region/ portal/ periportal region).	134
<u>15</u>	Comparison between group A2 and group B2 regarding SMA (in granuloma region/ portal/ periportal region).	134
<u>16</u>	Comparison between group A3 and group B3 regarding SMA (in granuloma region/ portal/ periportal region).	135
<u>17</u>	Comparison between group A1 and group A3 regarding SMA (in granuloma region/ portal/ periportal region).	136

List of Tables

<u>Table</u>	<u>Title</u>	<u>Page</u>
<u>1</u>	Variations of granuloma size in tested groups at the histopathological level.	111
<u>2</u>	Comparison of granuloma size between group A1 and group A3.	114
<u>3</u>	Mean number of granulomas in control and test groups.	115
<u>4</u>	Comparison of associated histological changes of intra-hepatic billiary radicals in control and test groups.	118
<u>5</u>	Comparison of associated histological changes of intra-hepatic billiary radicals in group A1 and group A3.	121
<u>6</u>	Variations of histologic changes at peri-granulomatous fibrosis (H&E-MT) in tested groups.	126
<u>7</u>	Comparison between group A1 and group A3 regarding peri-granulomatous fibrosis (PGF) (H&E-MT).	128
<u>8</u>	Variations of SMA immunostaining results (in granuloma region/ portal/ periportal region) in study groups.	133
<u>9</u>	Comparison of SMA (in granuloma region/ portal/ periportal region) between group A1 and group A3.	135
<u>10</u>	Biochemical tests results for group A3 and B3.	137