Effects of $^{137}\text{Cesium}$ chronic low dose exposure on neovascularization process

Dimassi S, Kereselidze D, Magneron V, Beugnies L, Sache A, Ebrahimian T.G

ICRR 2019
25-29 August 2019, Manchester
Study context

**Post-accident situations**

- Chernobyl incident (1986)
- Fukushima daiichi incident (2011)

Maps of cesium 137 remanent deposits at the regional / continental scale for Chernobyl (left) and Fukushima (right) incidents (source: www.IRSN.fr)

- Most abundant long-lasting radionucleid released
- 30 year half-life
- Ubiquitous in contaminated ecosystems

Chronic exposure to radionucleides by ingestion of contaminated food

Effects of ionizing exposure on physiological functions in long term
Epidemiological studies

High doses

Clearly established relation between high doses and cardiovascular disease occurrence

Irradiation of Hiroshima and Nagasaki bomb survivors (Wang et al., 1999)
Radiotherapy studies (Clarke, 2005; Hoving, 2007...)

Cardiovascular diseases
Epidemiological studies

Low doses

- Relationship not clear for low doses
  - Life span study
  - Mayak workers
  - Other environmental or professional cohorts

High doses

- Clearly established relation between high doses and cardiovascular disease occurrence
  - Irradiation of Hiroshima and Nagasaki bomb survivors (Wang et al., 1999)
  - Radiotherapy studies (Clarke, 2005; Hoving, 2007...)

Cardiovascular diseases
Study context

Immunomodulatory mechanisms

Neovascularization

Macrovascular injury

Microvascular injury

Atherosclerosis

Angiogenic deficit

Ischemia

Cardiovascular diseases
Study context

Chronic low doses → Immunomodulatory mechanisms → Neovascularization

- Macrovacular injury
- Atherosclerosis

- Microvascular injury
- Angiogenic deficit

Ischemia → Activation of defense mechanisms

- Proinflammatory factor expression
- Atheromatous lesion area and macrophage content

Cardiovascular diseases

Mitchel et al., 2011, Rad Res
Le Gallic et al., 2015, PlosOne
Mancusso et al., 2015, Oncotarget
Vieria et al., 2017
Ebrahimian et al., 2018, Rad Res
Study context

Chronic low doses → Immunomodulatory mechanisms → Neovascularization → Acute/ fractioned moderate doses

- Macrovascular injury
  - Atherosclerosis
- Microvascular injury
  - Angiogenic deficit

Ischemia

Cardiovascular diseases

→ Stimulation of neovascularization after vascular injury

- Blood perfusion after injury
- Circulating levels of proangiogenic factors (VEGF, PIGF, G-CSF...)
- Number of circulating Endothelial Progenitor Cells (EPCs)

Neovascularization stimulation via the nitric oxide (NO) pathway

Sonveaux et al., 2003, P Cancer Res
Heissig et al., 2005, JEM
Vala et al., 2010, PlosOne
Ministro et al., 2017, Cardiovasc Res
Study context

Chronic low doses

Immunomodulatory mechanisms

Macrovascular injury
Atherosclerosis

Neovascularization

Microvascular injury
Angiogenic deficit

Ischemia

Cardiovascular diseases

Acute/ fractioned moderate doses

Objective:

Study of effects of Cs chronic low dose exposure on neovascularization process and implication of the NO pathway
Proangiogenic factors (VEGF, PDGF, Ang-1...)

Neovascularization process

Activation of ECs in quiescent blood vessels

Angiogenesis

formation of new blood vessels from endothelial cells (ECs) of the existing vasculature

Sprouting angiogenesis

Proliferation, migration and differentiation

Vasculogenesis

formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)

EPCs recruitment from bone marrow

EPCs incorporation into the vasculature

EPCs from bone marrow

Mature network
Experimental design

6-month Cs contamination
C57BI/6

Ctrl
Cs20 kBq/L
Cs100 kBq/L

Proangiogenic factors
(VEGF, PDGF, Ang-1...)

Neovascularization process
(Angiogenesis + Vasculogenesis)

Angiogenesis
formation of new blood vessels from endothelial cells (ECs) of the existing vasculature

Vasculogenesis
formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)

Activation of ECs in quiescent blood vessels

EPCs recruitment from bone marrow

Proliferation, migration and differentiation

Sprouting angiogenesis

Mature network

EPCs incorporation into the vasculature

ICRR - Manchester - August 2019
Experimental design

6-month Cs contamination +/- LN

L-NAME (LN)

NOS

Proangiogenic factors (VEGF, PDGF, Ang-1...)

Neovascularization process

(Angiogenesis + Vasculogenesis)

Activation of ECs in quiescent blood vessels

Angiogenesis

formation of new blood vessels from endothelial cells (ECs) of the existing vasculature

Sprouting angiogenesis

Proliferation, migration and differentiation

EPCs recruitment from bone marrow

Vasculogenesis

formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)

EPCs incorporation into the vasculature

C57Bl/6

Ctrl

Cs20 kBq/L

Cs100 kBq/L

Ctrl + LN

Cs20 kBq/L + LN

Cs100 kBq/L + LN

ICRR - Manchester - August 2019
Experimental design

6-month Cs contamination +/- LN

1/ Post-ischemic neovascularisation

Hindlimb femoral artery ligation → ISCHEMIA

In vivo Cutaneous Blood flow measurement (Laser Doppler Imaging)

Neovascularization process

Angiogenesis
- Formation of new blood vessels from endothelial cells (ECs) of the existing vasculature
- Sprouting angiogenesis
- Activation of ECs in quiescent blood vessels

Vasculogenesis
- Formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)
- EPC proliferation and differentiation into endothelial cells
- EPCs recruitment from bone marrow

ICRR - Manchester - August 2019
Experimental design

6-month Cs contamination +/- LN

C57BI/6

Cs100 kBq/L +LN

1/ Post-ischemic neovascularisation

2/ Angiogenesis

Aorta prelevment

Aortic ring culture into matrigel

Sprouting analysis

8 days

ICRR - Manchester - August 2019
Experimental design

1/ Post-ischemic neovascularisation
2/ Angiogenesis
3/ Vasculogenesis
  3.a/ EPC migration & incorporation into matrigel

Histological analyses (Trichrome-Masson coloration)

Neovascularization process

(Angiogenesis + vasculogenesis)

Activation of ECs in quiescent blood vessels
Proliferation, migration and differentiation
Sprouting angiogenesis
Angiogenesis
formation of new blood vessels from endothelial cells (ECs) of the existing vasculature
Mature network

EPCs recruitment from bone marrow
EPCs incorporation into blood vessels
EPC proliferation and differentiation into endothelial cells
Vasculogenesis
formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)

ICRR - Manchester - August 2019
Experimental design

1/ Post-ischemic neovascularisation
2/ Angiogenesis
3/ Vasculogenesis
   3.a/ EPC migration & incorporation into matrigel
   3.b/ EPC tubular formation into matrigel

Bone marrow derived mononuclear cell isolation

Cell/Matrigel injection

Collection of matrigel

Histological analyses (Trichrome-Masson coloration)

Neovascularization process (Angiogenesis + vasculogenesis)

Angiogenesis
- formation of new blood vessels from endothelial cells (ECs) of the existing vasculature
- Sprouting angiogenesis
- Proliferation, migration, and differentiation

Vasculogenesis
- formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)
- EPC proliferation and differentiation into endothelial cells
- EPCs recruitment from bone marrow

Activation of ECs in quiescent blood vessels
1/ Post-ischemic neovascularisation

Results & Interpretation

**Cs contamination +/- LN**

- C57Bl/6
- Cs100 kBq/L + LN
- Cs20 kBq/L + LN

**In vivo Cutaneous Blood flow measurement (Laser Doppler Imaging)**

**Hindlimb femoral artery ligation**

**Measurement of cutaneous blood flow of ischemic/non ischemic hindlimb ratio**

- Ctrl
- Cs20
- Cs100

Increased hindlimb perfusion after Cs chronic exposure for Cs20 and Cs100 groups

n=15 mice/group

*p<0.05 vs Ctrl; **p<0.005 vs Ctrl
1/ Post-ischemic neovascularisation

Cs contamination +/- LN

Hindlimb femoral artery ligation

6 months

In vivo Cutaneous Blood flow measurement (Laser Doppler Imaging)

Measurement of cutaneous blood flow of ischemic/non-ischemic hindlimb ratio

Increased hindlimb perfusion after Cs chronic exposure for Cs20 and Cs100 groups. LN treatment abolished the increase in ischemia hindlimb flow for the Cs100LN group.
Increased sprouting angiogenesis after chronic exposure with Cs100
2/ Angiogenesis

Results & Interpretation

Cs contamination +/- LN

Aorta prelevment

Aortic ring culture into matrigel

Sprouting analysis

Week 1

Day 1

Day 8

Measurement of mean number of sprouts per aortic ring

Measurement of mean length of sprouts per aortic ring

N=10 rings/mouse, 2-3 mice/group
*p<0.05 vs Ctrl; **p<0.005 vs Ctrl; §p<0.05 vs CtrlLN

Increased sprouting angiogenesis after chronic exposure with Cs100
3.a/ Vasculogenesis: EPC migration & incorporation into matrigel

- **Cs contamination +/- LN**
- **Injection of SDF-1/Matrigel**
- **Matrigel collect**

**Histological analyses**
(Trichrome-Masson coloration)

**Histological section of matrigel/SDF-1 injected subcutaneously. Trichrome-Masson coloration**

**Importance**: No effect of Cs exposure on the EPC capacity to migrate from bone marrow and incorporate vessels

n=10 mice/group
3.a/ Vasculogenesis: EPC migration & incorporation into matrigel

Histological section of matrigel/SDF-1 injected subcutaneously. Trichrome-Masson coloration

No effect of Cs exposure on the EPC capacity to migrate from bone marrow and incorporate vessels

Reduction of EPC potency for Cs100LN group
3.b/ Vasculogenesis: EPC tubular formation into matrigel

**Results & Interpretation**

- **Cs contamination +/- LN**
- **Bone marrow derived mononuclear cell isolation**
- **Cell/Matrigel injection**
  - N=15/group
- **Matrigel collect**
- **Histological analyses (Trichrome-Masson coloration)**

**Histological section of matrigel injected subcutaneously and containing bone marrow derived mononuclear cell. Trichrome-Masson coloration**

**Evaluation of EPC ability to elaborate tubular structure into matrigel**

- **Scoring index**
  - **Ctrl**
  - **Cs 20 kbq/L**
  - **Cs 100 kbq/L**

**No effect of Cs exposure on EPC capacity to form tubular structure**

ICRR - Manchester - August 2019
3.b/ Vasculogenesis: EPC tubular formation into matrigel

- **Cs contamination +/- LN**
  - Bone marrow derived mononuclear cell isolation
  - Cell/Matrigel injection
  - Matrigel collect
  - Histological analyses (Trichrome-Masson coloration)

- **Evaluation of EPC ability to elaborate tubular structure into matrigel**

- **No effect of Cs exposure on EPC capacity to form tubular structure**
- **No effect of LN traitement**
Cs chronic low dose contamination

- Stimulation of post-ischemic neovascularization
- Enhancement of sprouting angiogenesis

**Cs**100 kBq/L

6 months

**Neovascularization process**

**Angiogenesis**
- formation of new blood vessels from endothelial cells (ECs) of the existing vasculature
- Sprouting angiogenesis
- Activation of ECs in quiescent blood vessels

**Vasculogenesis**
- formation of new blood vessels through the recruitment of bone marrow-derived endothelial progenitor cells (EPCs)
- EPCs proliferation and differentiation into endothelial cells
- EPCs recruitment from bone marrow
- EPCs incorporation into blood vessels

**ICRR - Manchester - August 2019**
**Conclusion**

**Cs chronic low dose contamination**

- Stimulation of post-ischemic neovascularization
- Enhancement of sprouting angiogenesis

Implication of the NO pathway

Effects on vasculogenesis via the NO pathway?
→ Evaluation of capillary density of hindlimb skeletal muscles (immunohistochemistry)

→ Analyse of molecular expression pattern of principal factors implicated in neovascularization process
Acknowledgments

LRTOX laboratory

CHRONEO Team

Ebrahimian Teni G
Kereselidze Dimitri
Magneron Victor
Beugnies Louison
Sache Amandine
Daily chronic intake:

75-90 Bq/day/animal
3.2 kBq/kg/day

Bertho et al., 2010
Synhaeve et al., 2011
Le Gallic et al., 2015

12 to 15 years following Chernobyl incident, in zone II (near Narodici, 75km west Chernobyl)

Daily chronic intake of populations living in countries contaminated by the Chernobyl accident

Handl et al., 2003
<table>
<thead>
<tr>
<th>Cs concentration</th>
<th>Cumulative absorbed dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 kBq/L</td>
<td>15 mGy</td>
</tr>
<tr>
<td>100 kBq/L</td>
<td>75 mGy</td>
</tr>
</tbody>
</table>

Dose rate: 6 μGy/h
In vivo zooradiometric measurement

Energie (Kev): 661.66

Activité (Bq/mg)
Arterial pressure measurement

500 µg LN/ml
250 mg LN/kg/day

6 months

Mean arterial pressure (MAP)

MAP (mm Hg)

Ctrl  Cs20  Cs100  CtrlLN  Cs20LN  Cs100LN
Chronic low-dose contamination
ApoE-/- 137Cs at 0, 4, 20 or 100 kBq/L
6 months
Cardiovascular diseases
Atherosclerosis
Angiogenic deficit
Macrovascular injury
Atheromatous lesion area
Macrophage content in atheromatous plaques and proinflammatory factor expression
Le Gallic et al., 2015, PlosOne
Immunomodulatory mechanisms
Neovascularization
Microvascular injury
Immuno-modulatory
Vascular integrity
Immunomodulatory mechanisms

Vascular integrity

Neovascularization

Macrovascular injury

Atherosclerosis

Microvascular injury

Angiogenic deficit

Ischemia

Cardiovascular diseases

Induced ischemia

Hindlimb ischemia

Irradiation

0.3 Gy x 4

Blood perfusion after ischemia stimulating neovascularization

Circulating levels of proangiogenic factors

Number of circulating Endothelial Progenitor Cells

Ministro et al., 2017, Cardiovasc Res

Vala et al., 2010, PlosOne

Hindlimb ischemia

Revascularization after ischemia

VEGF product

Heissig et al., 2000

Inter-ray vessel density

0 Gy 0.5 Gy

Angiogenesis via the VEGF pathway

16 days after amputation

0 Gy 0.5 Gy

Hindlimb revascularization after ischemia

MMP-9+/+

MMP-9+/+

Laser Doppler

vascularization via the VEGF pathway

Ministro et al., 2017, Cardiovasc Res

IRS